

The enigma of lymph node staging in breast cancer

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The enigma of lymph node staging in breast cancer

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The enigma of lymph node staging in breast cancer

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Chapter 1

Introduction

Introduction

Breast cancer is the most common type of invasive cancer among women worldwide, with an estimated 1.7 million cases and 521,900 deaths in 2012.¹ In the Netherlands, more than 14,000 patients are newly diagnosed with breast cancer each year.² Nowadays, standard treatment consists of an operation of the breast and axilla, complemented with radiation and/or systemic therapy. Obviously, survival and reducing recurrence are the most important goals. Due to this extensive treatment, mean five year overall survival has increased over the last decades to 90%.³ The in general excellent survival caused an attention shift from merely survival to the presence of morbidity and quality of life after breast cancer treatment. Better patient selection for specific therapies and use of less invasive techniques, in order to avoid overtreatment on an individual level, aim to further minimize morbidity and complications of therapy.

Axillary lymph nodes are examined at breast cancer diagnosis, because of their prognostic relevance. Axillary lymph node dissection (ALND), i.e. the complete removal of all axillary lymph nodes, used to be the surgical treatment of choice to provide accurate information about actual lymph node status for a long period. Eventually, approximately 30-40% of all patients had axillary lymph node metastases.⁴ Hence, 60-70% of all patients underwent ALND without having any clinical or prognostic benefit of the surgical procedure. However, ALND causes long-term morbidities in up to 45% of patients, such as lymphedema, nerve injury and reduced shoulder function.⁵ A less invasive procedure to identify malignant lymph nodes was therefore needed.

In 1994 sentinel lymph node biopsy (SLNB) was introduced as a less invasive technique to evaluate the axillary lymph node status in clinically node negative patients.⁶ The principle of SLNB relies on the lymphatic drainage from the primary tumour in the breast to one or more regional lymph nodes, assuming that if these lymph nodes contain no tumour cells, all other regional lymph nodes can be considered benign. In case of sentinel lymph node metastases, a completion ALND was performed. Several studies confirmed the safety and clinical applicability of SLNB as less invasive procedure for nodal staging in clinically node negative patients.^{7,8} Yet, complications of SLNB still occur, like lymphedema, numbness and pain, albeit less frequently than in ALND.⁹

In the Netherlands, standard axillary examination consists of an ultrasound (US) with tissue sampling (if indicated) to identify clinically node positive patients.^{10,11} A previous study demonstrated that US can identify less than 50% of the patients with axillary lymph node metastases.¹² Yet, in patients who are

considered clinically node positive (based on US findings) ALND is performed directly, which prevents these patients undergoing two surgical procedures (SLNB followed by completion ALND).

The hypothesis of detecting every positive lymph node in order to improve prognosis was reconsidered in 2011. The ACOSOG Z0011 trial, a randomized clinical trial which included clinically node negative breast cancer patients (based on physical examination) treated with breast conserving therapy, demonstrated that in case of one or two positive lymph nodes after sentinel lymph node biopsy there was no prognostic benefit of performing a completion axillary lymph node dissection or not.^{13,14} These results were confirmed by the IBCSG 23-01 and AATRM 048/13/2000 trials in which patients with one to three positive nodes were investigated.^{15,16} In summary, excluding advanced axillary nodal disease (i.e. more than three positive nodes) rather than detecting clinically positive nodes is becoming more and more important. Previous studies have demonstrated that axillary US is an excellent imaging modality to exclude advanced axillary nodal disease in case of negative findings.^{12,17} However, in case of positive findings US is unable to differentiate between limited (i.e. one to three positive nodes) and advanced axillary nodal disease.¹²

If a non-invasive technique would be able to accurately differentiate between patients with and without axillary lymph node metastases, axillary surgery could be safely omitted in case of node negative findings. This would be of added value for many patients, as SLNB and its subsequent morbidity could be omitted in all node negative patients then. Current imaging modalities like US, magnetic resonance imaging (MRI) and positron emission tomography/computed tomography (PET/CT) are insufficiently accurate to differentiate between node negative and positive findings, with previously reported percentages of 25% (=52/208), 24% (82/337), and 47% (=42/90) of patients still containing axillary lymph node metastases in case of negative findings.¹⁷⁻¹⁹ Regarding MRI for axillary nodal staging, diagnostic performance improved with the use of a dedicated axillary MR protocol.²⁰ In addition, a feasibility study concerning gadofosveset-enhanced dedicated axillary MRI in breast cancer patients demonstrated promising results for axillary nodal staging, with a reported sensitivity of 86%, specificity of 94%, positive predictive value (PPV) of 75% and negative predictive value (NPV) of 97%, on a node-by-node analysis.²¹ However, these results were based on a small sample size of just ten patients, which demands further studies to confirm these results.

Since 1958 the definitive histopathological result of surgical treatment in breast cancer patients is classified according to the TNM classification.²² This

staging system defines the extent of primary tumour (T status), the extent of nodal disease (N status), and the presence of distant metastases (M status). The TNM classification is regularly updated by the International Union against Cancer, with a view to maintain a clinically relevant classification system.²³ The N status represents the number, size and location of regional lymph node metastases. In clinical practice, TNM classification is often used to decide which type of additional treatment patients will receive. For instance radiotherapy indications and the use of adjuvant systemic therapy, can be decided based upon these results.²⁴ The existence of such a dedicated classification system underlines the importance of accurate clinical lymph node staging prior to therapy initiation in breast cancer patients.

Besides irradiation and surgical treatment, systemic therapy has an important role in treatment of breast cancer patients. Since the introduction of chemotherapy in the 20th century, administration of chemotherapy has shown substantial improvement in patient's prognosis.^{24,25} The indication for systemic therapy depends on several criteria, for instance tumour biology, size, grade and presence and number of lymph node metastases.²⁶ Earlier, systemic therapy was applied after the operation of breast and axilla, the so called 'adjuvant setting'. In recent years, systemic therapy is more and more administered prior to surgery, i.e. 'neoadjuvant setting'.^{27,28} This might result in regression of tumour size or even a pathological complete response (pCR, i.e. no residual tumour present after treatment). Neoadjuvant systemic therapy might result in smaller breast-conserving therapy, breast-conserving therapy where initially mastectomy was preferred, as well as in vivo tumour response monitoring.^{29,30} Neoadjuvant systemic therapy allows axillary lymph node metastases to convert to axillary pCR as well, making the ALND a redundant operation. According to recent studies, axillary pCR appears in 20–60% of all clinically node positive patients, depending on tumour biology.³¹ Furthermore, identification of patients whose axillary lymph node metastases converted to axillary pCR is important since it is a sign of extreme chemosensitivity.^{32,33} So far, no non-invasive technique has shown accurate results to discriminate axillary pCR from remaining lymph node metastases after neoadjuvant systemic therapy.^{34,35} A less invasive technique as opposed to ALND is therefore warranted in order to identify axillary pCR in clinically node positive patients treated with neoadjuvant systemic therapy.

Aims and outline of this thesis

The overall aim is to investigate the enigma of lymph node staging in breast cancer patients, by improving the diagnostic accuracy of nodal staging in order to achieve a more patient-tailored treatment with minimal invasive therapy and reduced morbidity. This thesis consists of three parts concerning lymph node staging in breast cancer: invasive, non-invasive and classification.

Part I – Invasive lymph node staging

Chapter 2 is a meta-analysis on the sentinel lymph node biopsy as axillary staging technique in clinically node positive patients treated with neoadjuvant systemic therapy. *Chapter 3* describes the RISAS-procedure (defined as a combination of the sentinel lymph node biopsy and an iodine seed placement and removal in the pathologically confirmed axillary lymph node metastasis) for axillary staging in clinically node positive breast cancer patients after neoadjuvant systemic therapy.

Part II – Non-invasive lymph node staging

Chapter 4 investigates the diagnostic performance of standard T2 weighted breast MRI compared to axillary US to differentiate between patients with no (pNo), limited (pN1) and advanced (pN2-3) axillary nodal disease. In *chapter 5* a validation study examines the diagnostic performance of gadofosveset-enhanced axillary MRI in breast cancer patients. *Chapter 6* assesses the added value of dedicated axillary hybrid ¹⁸F-FDG PET/MRI for lymph node staging in clinically node positive breast cancer patients.

Part III – Classification of lymph node staging

Chapter 7 evaluates the classification of breast cancer patients with a pN3a nodal status (at least one infraclavicular lymph node metastases or ten or more axillary lymph node metastases). In *chapter 8* the prognosis and classification of residual axillary disease (isolated tumour cells, micrometastases or macrometastases) is studied in clinically node positive breast cancer patients treated with neoadjuvant chemotherapy. The TNM classification of solitary internal mammary lymph node metastases is examined in *chapter 9*.

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Part I

Invasive lymph node staging

Chapter 2

The diagnostic performance of sentinel lymph node biopsy in pathologically confirmed node positive breast cancer patients after neoadjuvant systemic therapy: A systematic review and meta-analysis

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ML Smidt

Eur J Surg Oncol. 2015;41(10):1278-1287

Abstract

Purpose

To provide a systematic review and meta-analysis of studies investigating sentinel lymph node biopsy after neoadjuvant systemic therapy in pathologically confirmed node positive breast cancer patients.

Methods

Pubmed and Embase databases were searched until June 19th, 2015. All abstracts were read and data extraction was performed by two independent readers. A random-effects model was used to pool the proportion for identification rate, false-negative rate (FNR) and axillary pCR with 95% confidence intervals. Subgroup analyses affirmed potential confounders for identification rate and FNR.

Results

A total of 997 abstracts were identified and eventually eight studies were included. Pooled estimates were 92.3% (90.8-93.7%) for identification rate, 15.1% (12.7-17.6%) for FNR and 36.8% (34.2-39.5%) for axillary pCR. After subgroup analysis, FNR is significantly worse if one sentinel node was removed compared to two or more sentinel nodes (23.9% versus 10.4%, $P=0.026$) and if studies contained clinically nodal stage 1-3, compared to studies with clinically nodal stage 1-2 patients (21.4 versus 13.1%, $P=0.049$). Other factors, including single tracer mapping and the definition of axillary pCR, were not significantly different.

Conclusion

Based on current evidence it seems not justified to omit further axillary treatment in every clinically node positive breast cancer patients with a negative sentinel lymph node biopsy after neoadjuvant systemic therapy.

Introduction

The timing of administration of systemic therapy in breast cancer patients has undergone major changes over the last decade. Chemotherapy and immunotherapy are increasingly administered in neoadjuvant rather than adjuvant setting.¹ This approach allows in vivo tumour response evaluation to systemic therapy. The downsizing effect of systemic treatment on the primary tumour can facilitate breast conservation therapy where mastectomy would have been indicated initially.^{2,3}

The increased use of neoadjuvant systemic therapy creates new clinical dilemmas, especially regarding the nodal staging work-up. In clinically node negative patients it seems appropriate to perform sentinel lymph node biopsy after neoadjuvant systemic therapy.⁴ The current standard for clinically node positive patients is still an axillary lymph node dissection after completion of neoadjuvant therapy. Studies however have reported axillary pathological complete response (pCR) in 20-42% of initially node positive patients.⁵⁻⁸ Therefore performing sentinel lymph node biopsy after neoadjuvant systemic therapy could potentially identify the true node negative patients and prevent unnecessary axillary lymph node dissection.⁹

Many studies have been published on the diagnostic performance of sentinel lymph node biopsy after neoadjuvant systemic therapy in patients with clinically node positive breast cancer, reporting conflicting outcomes regarding the identification rate of a sentinel lymph node and the false-negative rate (FNR) of the procedure.

Our study aim was to investigate the accuracy of sentinel lymph node biopsy in identifying axillary pCR in pathologically confirmed node positive breast cancer patients receiving neoadjuvant systemic therapy by providing a systematic review and meta-analysis of all relevant studies.

Methods

Literature search strategy

For this study PubMed (including Medline) and Embase databases were searched until June 19th, 2015. Free text and Medical Subject Headings (MeSH) terms were used for breast cancer, sentinel lymph node biopsy, diagnostic performance, lymph nodes and neoadjuvant systemic therapy. Full search strategies are provided in Appendix 2.1.

Study inclusion criteria

Studies were eligible if accuracy of sentinel lymph node biopsy was evaluated after neoadjuvant systemic therapy in pathologically confirmed node positive breast cancer patients.

Studies had to meet the following inclusion criteria: 1) patients underwent both sentinel lymph node biopsy and axillary lymph node dissection, 2) clinically node positive status was verified by histological examination prior to start neoadjuvant systemic therapy, 3) the nodes retrieved from sentinel lymph node biopsy and axillary lymph node dissection were analyzed by standard institutional guidelines for histological examination, 4) reported data enabled construction of 2 x 2 contingency tables with absolute numbers of true positive (TP), false negative (FN) and true negative (TN) test results.

Only papers written in English, German, French, Spanish or Dutch language were included. Case reports, technical notes, comments on earlier publications, editorials, conference proceedings, reviews or meta-analyses were excluded.

Studies with duplicated results from other studies were excluded. Two independent readers read all abstracts to determine eligibility. Full text articles were read for all potential eligible studies. These readers also decided which studies met the predefined inclusion criteria. In case of discrepancies, consensus was reached by these two readers. Quality assessment of included articles was performed by using the QUADAS-2 tool.¹⁰

Data extraction

From studies which met the inclusion criteria, the two independent readers extracted the following predefined parameters: first author, year of publication, study design, sample size, type of primary tumour, clinical tumour and nodal stage, neoadjuvant systemic therapy regimen, the use of immunohistochemistry on axillary nodes, mapping method of sentinel node and definition of pathologic complete axillary response. Data were also extracted on relevant outcome parameters: identification rate, axillary pCR and the false negative rate (FNR) of sentinel lymph node biopsy.

Identification rate was defined as the percentage of patients in which a sentinel node could be identified during surgery divided by all patients in whom a sentinel node procedure was undertaken. Axillary pCR was defined as absence of cancer according to histological diagnosis after axillary lymph node dissection.

Data from patients with successfully identified sentinel nodes were used to construct 2 x 2 contingency tables for each study separately. Since false positive cases will not occur, only true positive (TP), false negative (FN) and true negative (TN) values were listed. FNR, the complement of sensitivity, was determined as

FN/(TP + FN) and NPV was determined as TN/(FN + TN). The percentage of patients with axillary pCR was defined as TN/(TP + FN + TN).

Statistical analysis

Statistical analyses were performed in STATA 11.2 (StataCorp LP, Texas, USA) and SPSS (version 22, IBM, Armonk, New York, USA).

Random-effects models for meta-analysis were used to calculate pooled proportions for identification rate, FNR and axillary pCR rate with 95% confidence intervals (CI).¹¹ Forest plots show variation in study results and the pooled estimates. Statistical heterogeneity was tested with chi-squared test and was quantified by I²-index. Publication bias was evaluated by funnel plots and by the Egger's test for funnel plot asymmetry. In absence of a relation between sample size and effect size, the study estimates will form a symmetrical funnel shape around the pooled estimate, points from small studies being more dispersed than point from larger studies. Asymmetry indicates an association between sample size and effect size and may be caused by publication bias, but there can also be other causes.¹²

Subgroup analyses were performed to evaluate the impact of several factors on identification rate and FNR. Factors that were considered relevant were year of publication, study design, sample size, clinical nodal stage at presentation, definition of axillary pCR and additional immunohistochemistry. Linear regression analyses were used to correlate FNR with identification rate and FNR with axillary pCR. Statistical significance was considered as *P*-values (two-sided) <0.05.

Results

Search strategy and study selection

The literature search identified 1146 publications. After removing 149 duplicates, the reading of 997 abstracts of the remaining papers resulted in the selection of 28 eligible papers. Full text of all these papers was read. Finally, eight articles were considered to meet the inclusion criteria and were included for final review (Figures 2.1 and 2.2).

General study characteristics

In these eight different studies, a total of 1395 patients participated (range 61-687).¹³⁻²⁰ Initial node positive disease was either cytologically or histologically proven for all patients at initial diagnosis. Neoadjuvant systemic therapy regimens

varied within studies. Sentinel lymph node procedure was followed by complete axillary lymph node dissection in all studies. Table 2.1 shows a detailed overview of all study characteristics. Table 2.2 shows an overview of all contingency tables with absolute numbers of the selected studies (including the NPVs of all studies).

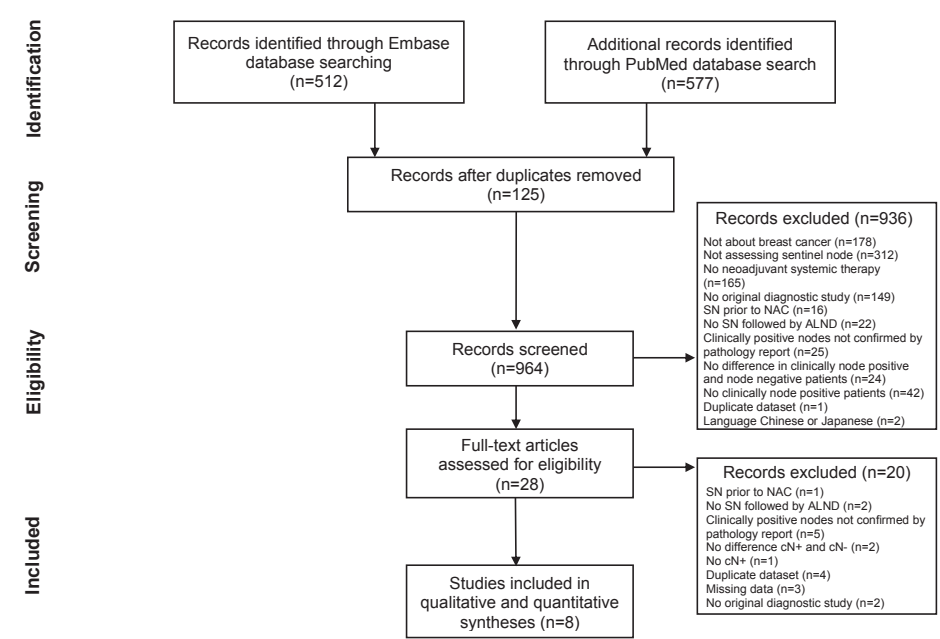


Figure 2.1 Flow diagram of preferred reporting items for systematic reviews and meta-analyses (PRISMA).

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Boileau	⊕	⊕	⊕	⊕	⊕	⊕	⊕
Boughey	⊕	⊕	⊕	⊕	⊕	⊕	⊕
Brown	⊗	⊕	⊕	⊕	?	⊕	⊕
Kang	⊕	⊕	⊕	⊕	⊕	⊕	⊕
Ozmen	⊕	⊕	⊕	⊕	⊕	⊕	⊕
Park	⊗	⊕	⊕	⊕	?	⊕	⊕
Shen	⊗	⊕	⊕	⊕	⊕	⊕	⊕
Yagata	⊕	⊕	⊕	⊕	⊕	⊕	⊕

⊕ = Low risk, ⊗ = High risk, ? = Unclear risk

Figure 2.2 QUADAS-2 tool. Evaluation of risk of bias and applicability of primary diagnostic accuracy studies.

Table 2.1 Overview of studies included.

Author	Year	Study design	Sample size	cT-stage	Breast cancer subtypes (%)	cN-stage	Regimen neoadjuvant systemic therapy (%)	Mapping method	IHC Lymph nodes	Definition of pCR
Boileau	2015	P	145	0-3	-	1-2	- AT (96) - A (1) - T (2)	2,3	Yes	Absence of isolated tumour cells, micro- and macrometastases
Boughey	2013	P	687	0-4	IDC (88) ILC (5) Other (7)	1-2	- AT (75) - A (6) - T (17)	1,2,3	No	Absence of micro- and macrometastases
Brown	2010	R	86	1-3	-	1-3	-	1,2,3	No	Absence of micro- and macrometastases
Kang	2011	R	66	0-3	DC (95) Other (5)	1-3	- AC (53) - AT (42)	1,2,3	Yes	Absence of any malignancy
Ozmen	2010	R	77	1-4	IDC (74) Other (26)	1-2	- FAC* - AC*	3	Yes	Absence of any malignancy
Park	2013	R	178	1-3	DC (96) Other (4)	1-3	- A (5) - AT (88)	1	Yes	Absence of micro- and macrometastases
Shen	2007	R	61	1-4	IDC (84) ILC (6) Other (10)	1-3	- FAC and T (59) - FAC (22) - T (7) - AT (4) - FEC, T and H (4) - T, capecitabine and FEC (1) - AT and oblimersen (1)	1,2,3	No	-
Yagata	2013	P	95	0-4	IDC (99) Other (1)	-	- FEC and T (76) - TAC (6) - FEC (2) - FEC, T and H (13) - TC (3)	3	Yes	Absence of micro- and macrometastases

Abbreviations: - Unknown, * Exact percentages missing. *Study design:* P = prospective, R = retrospective. *Breast cancer subtypes:* IDC = invasive ductal carcinoma, ILC = invasive lobular carcinoma, DC = ductal carcinoma. *Regimen neoadjuvant systemic therapy:* AT = antracycline and taxane, A = antracycline, T = taxane, AC = antracycline and cyclophosphamide, FAC = 5-Fluorouracil, antracycline and cyclophosphamide, FEC = 5-Fluorouracil, epirubicin and cyclophosphamide, H = trastuzumab (Herceptin), TC = taxane and cyclophosphamide. *Mapping methods:* 1 = radioactive isotope alone, 2 = blue dye alone, 3 = combination of blue dye and radioactive isotope.

Table 2.2 Overview contingency tables with absolute numbers of studies included.

Author	Identification Rate (%)	Sample size [†]	True and false positive	False negative	True negative	False-negative rate (%)	Negative predictive value (%)	Axillary pCR rate (%)
Boileau	87.6 (127/145)	127	76	7	44	8.4	86.3	34.6
Boughey	92.7 (637/687)	637	326	56	255	14.7	82.0	40.0
Brown	* 87.9 (58/66)	86	47	13	26	21.7	66.7	30.2
Kang		58	34	7	17	17.1	70.8	29.3
Ozmen	92.2 (71/77)	71	44	7	20	13.7	74.1	28.2
Park	94.9 (169/178)	169	78	22	39	22.0	75.8	40.8
Shen	91.8 (56/61)	56	30	10	16	25.0	61.5	28.6
Yagata	85.3 (81/95)	81	43	8	30	15.7	78.9	37.0

[†] Sample size with successfully identified sentinel node(s) during surgery. *No identification rate possible, patients were included if sentinel node was identified.

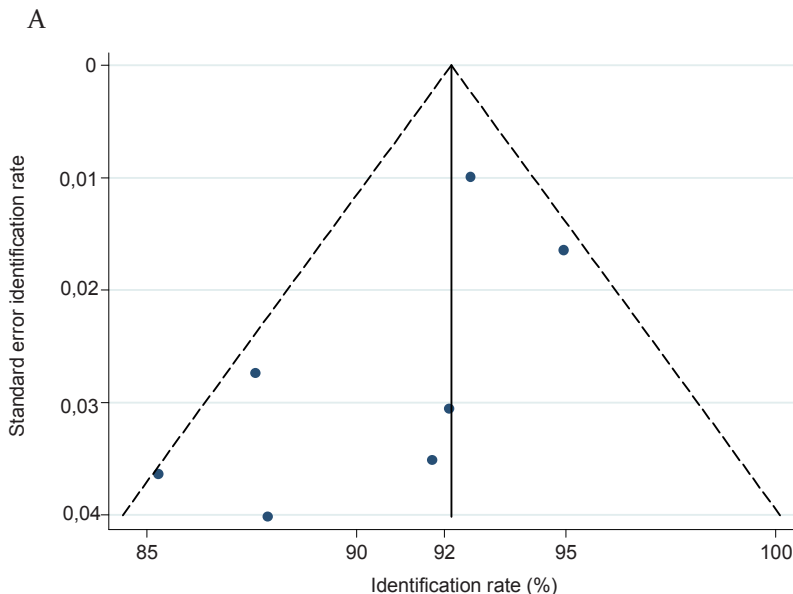
Sentinel lymph node biopsy

Identification rate

One of the included studies could not be used for the assessment of identification rate, because it was not reported.¹⁵ Figure 2.3 shows the Forest plot and funnel plot for study results. The pooled identification rate was 92.3% (95% CI, 90.8-93.7%). The funnel plot indicates a lower identification rate in studies with small sample size. The Egger's test resulted in an intercept of 1 and $P=0.120$.

Four studies subdivided their populations according to their different mapping methods for the identification of the sentinel lymph node during surgery: radioactive isotope alone, blue dye alone and the combination of both techniques.^{14,17,19-21} After subgroup analysis, the identification rates were respectively 93.9% (95% CI, 91.2-96.7%) for radioactive isotope alone, 78.8% (95% CI, 64.9-92.7%) for blue dye alone and 93.0% (95% CI, 91.2-94.8%) for the combination of both techniques. There were no statistical significances between blue dye and radioactive isotope alone ($P=0.18$), and blue dye and the combination of both techniques ($P=0.151$).

Further subgroup analyses showed no significant difference for year of publication, study design, sample size and clinical nodal stage (Appendix 2.2).



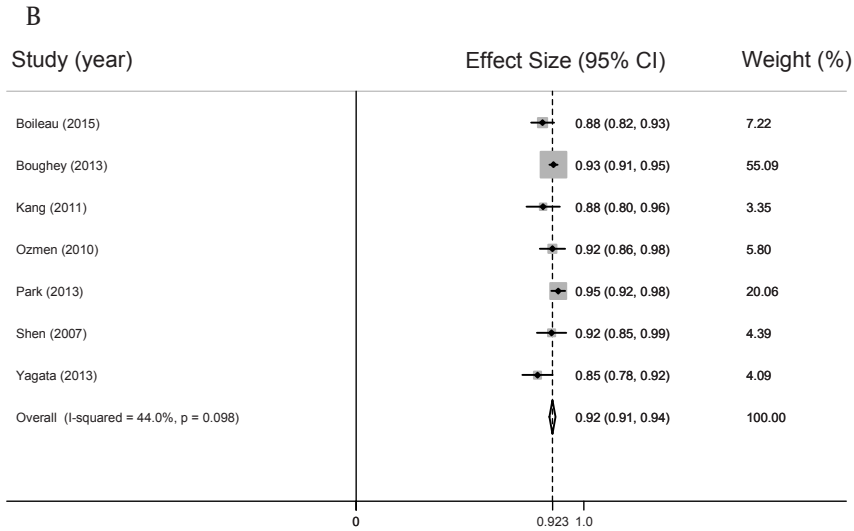


Figure 2.3 Forest plot (A) and funnel plot (B) with pseudo 95% confidence intervals of identification rate. The pooled identification rate was 92.3% ($I^2=44.0\%$, $P=0.098$).

False-negative rate

Figure 2.4 shows the Forest plot and funnel plot for study results. The pooled FNR was 15.1% (95% CI, 12.7-17.6%) with an I^2 index of 38.8%. The funnel plot for FNR shows that studies with small sample size tended to report higher FNRs than studies with larger sample size. The Egger's test resulted in an intercept of 1 and $P=0.224$. Figure 2.5 shows the correlation between FNR and identification rate of the included studies ($P=0.290$). There was no correlation between FNR and axillary pCR ($P=0.714$).

Two of the included studies used non-invasive restaging after neoadjuvant systemic therapy to identify clinically No patients after neoadjuvant systemic therapy, with the objective to perform the sentinel lymph node biopsy only in clinically No patients.^{17,20} Restaging consisted of physical examination, mammography, ultrasound or breast MRI. According to subgroup analysis, the FNR of these two studies was 14.7% (95% CI 7.8-21.5%), which was comparable to FNR of 15.2% (95% CI 12.6-17.8%) for all other studies ($P=0.885$).

Four studies subdivided their populations in patients with only one sentinel node removed during surgery versus patients with two or more sentinel nodes removed during surgery.^{13,14,18,20} After subgroup analysis in these studies, FNR was significantly higher if only one sentinel node was removed compared to two or more sentinel nodes with a pooled FNR of 23.9% (95% CI 17.2-30.6%) versus 10.4% (95% CI 7.7-13.2%) ($P=0.026$).

Further subgroup analysis showed a significantly higher FNR for studies which included patients with clinically nodal stage 1-3, compared to studies which only contained patients with clinically nodal stage 1-2 (21.4 versus 13.1%, $P=0.049$). Moreover, a prospective study design showed a trend towards significant improvement of FNR (13.3 versus 19.6%, $P=0.079$, respectively) (Appendix 2.2).

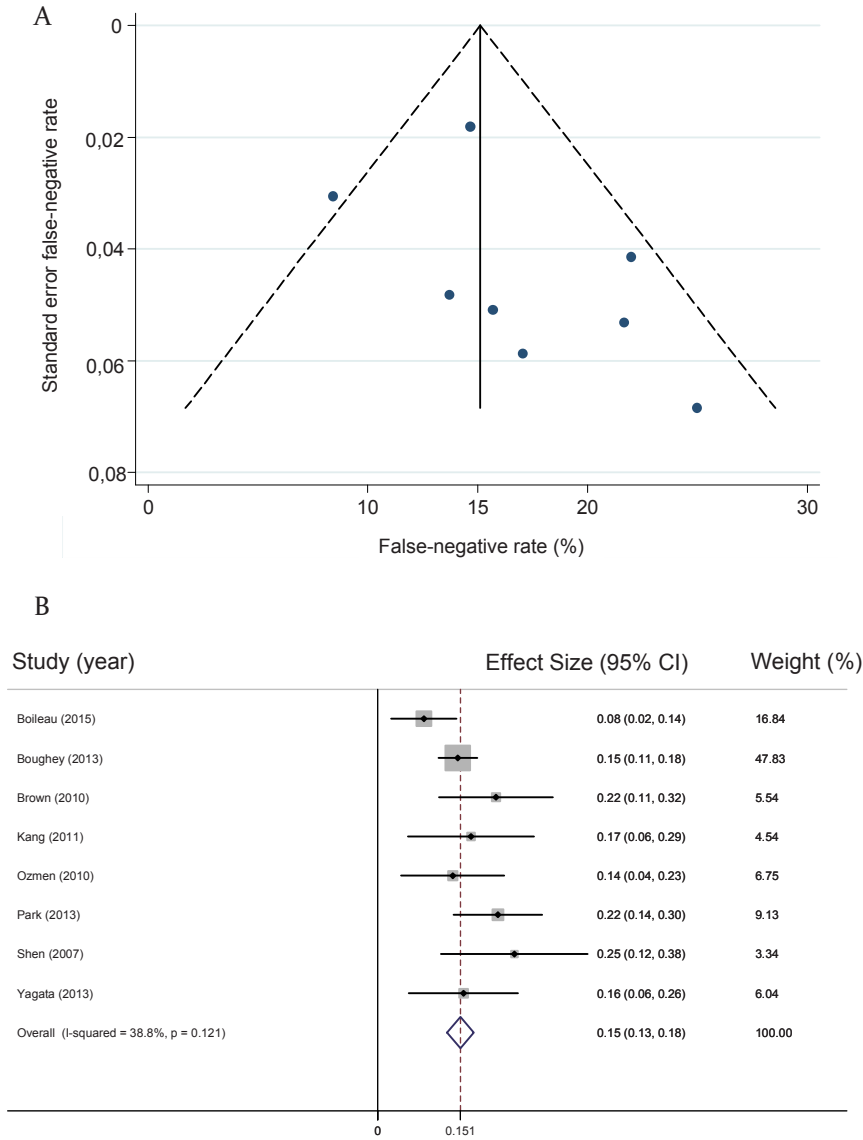


Figure 2.4 Forest plot (A) and funnel plot (B) with pseudo 95% confidence intervals of false-negative rate. The pooled false-negative rate was 15.1% ($I^2=38.8\%$, $P=0.120$).

Response evaluation

Axillary pathological complete response

In absolute numbers, 477 out of 1395 initially node positive patients achieved axillary pCR to neoadjuvant systemic therapy. Appendix 2.3 shows the Forest plot and funnel plot for study results. The pooled axillary pCR rate was 36.8% (95% CI, 34.2-39.5%) with an I^2 index of 41.5%. The funnel plot for axillary pCR shows that studies with small sample size tended to report lower pCRs than studies with larger sample size. The Egger's test resulted in an intercept of 1 and $P=0.102$.

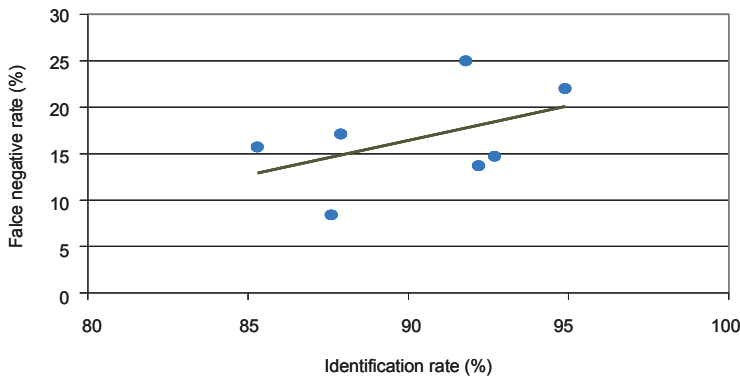


Figure 2.5 False-negative rate as function of identification rate. Increase of false-negative rate is associated with an increase of identification rate ($P=0.290$).

Discussion

This meta-analysis provides an overview of the diagnostic performance of the sentinel lymph node biopsy after neoadjuvant systemic therapy in pathologically confirmed initially node positive breast cancer patients. Our study showed a pooled identification rate of 92.3% and a pooled FNR of 15.1%.

Axillary lymph node dissection is currently the standard practice in clinically node positive breast cancer patients.⁵ It is associated with severe side effects in 45% of its patients, such as lymphedema, neuropathy and functional limitations.²² Clinically node positive patients converting to node negative status after neoadjuvant systemic therapy could potentially benefit from omitting an axillary lymph node dissection thus increasing the quality of life of breast cancer survivors. A prerequisite is a diagnostic test that can accurately select the patients with axillary pCR.

The success of identifying the sentinel lymph node during surgery in clinically node negative patients is inferior after neoadjuvant systemic therapy compared to primary surgery.²³ Despite the use of dual tracer mapping as opposed to single tracer mapping in sentinel lymph node biopsy, identification rate in clinically node negative patients remains inferior (95 versus 98%).²⁴ We hypothesize that both the presence of axillary metastases and the effect of the systemic treatment may contribute to changes in the lymphatic flow.²¹ For this meta-analysis, the use of blue dye alone as single tracer mapping agent showed a lower identification rate, compared to radioactive isotope alone or the combination of both techniques (78.8% versus 93.3 and 93.0%), however it was not statistically significant due to the small sample size of patients who received blue dye alone for tracer mapping (n=33 for blue dye alone, compared to n=301 for radioactive isotope alone and n=774 for dual tracer mapping).

According to the studies of Harlow et al.²⁵ and Kim et al.,²⁶ sentinel lymph node biopsy with a FNR of 10% is accepted as oncologically safe to avoid axillary lymph node dissection in clinically node negative patients undergoing primary surgery. Based on these results, recent studies in clinically node positive patients treated with neoadjuvant systemic therapy used a cut-off value of 10% for FNR.^{13,14} The use of this cut-off value of 10% is arbitrary. Maybe a FNR of 5% or even 15% would be more appropriate. Patients with a false negative sentinel lymph node biopsy are theoretically at risk to develop regional (nodal) recurrences, with reduced disease-free and overall survival. However, omission of axillary lymph node removal in clinically node negative patients does not affect survival according to the ACOSOG Z0011 trial.²⁷ In clinically node positive patients undergoing neoadjuvant systemic therapy, we assume patients will still receive adjuvant trastuzumab, radiotherapy and/or endocrine therapy on indication. Obviously, all patients already received appropriate chemotherapy. For these patients, the role of regional axillary radiotherapy to prevent regional recurrences will be investigated in the NSABP B-51 trial and the Alliance 11202 trial, both registered with Clinicaltrials.gov (NCT01872975 and NCT01901094). These trials will give us the final information on the safety of the omission of further axillary treatment in clinically node positive patients with a negative sentinel node after neoadjuvant systemic therapy.

We observed that FNR is mainly influenced by four conditions in clinically node positive or negative sentinel lymph node studies. First, FNR in clinically node negative patients is worse in whom only one sentinel node could be identified during surgery, compared to two or more sentinel nodes (14.3% versus 4.3%).²⁸ In this meta-analysis four studies investigated the difference between one and two or more sentinel nodes removed per patient. Subgroup analysis reported a higher FNR if one instead of two or more sentinel nodes were removed during

surgery (23.9% versus 10.7%, $P=0.026$, respectively).^{14,18} Although this finding seems promising, in clinical practice it is impossible to predict the number of sentinel nodes identified per patient during surgery. So far, no studies have investigated the value of additional node picking in case only one or two sentinel nodes are identified.

Second, extensive evaluation of the sentinel lymph node using immunohistochemistry seemed to reduce the FNR, from 13.3 to 8.4%.¹³ However, it is unclear in how many patients the sentinel lymph node contained isolated tumour cells, while the complementary axillary lymph node dissection revealed no additional metastases, thereby introducing possible overtreatment.¹³ Since, the importance of the detection of isolated tumour cells as indicator to decide whether or not to perform a complementary axillary lymph node dissection is unclear.

Third, FNR seems to be associated with the identification rate ($P=0.290$) (Figure 2.5). One would expect a reduction of FNR when the identification rate increases, suggesting performance of a more experienced surgeon during the sentinel lymph node biopsy.²⁹ However, according to our meta-analysis this is questionable.

Finally, the number of patients converting to axillary pCR in initially node positive patients can influence the FNR. If the amount of true negative patients reduces, for instance in low grade hormone-receptor positive, HER2-negative breast cancer patients treated with neoadjuvant systemic therapy with a reported pCR of 7.5% for both primary tumour and axilla,³⁰ both FNR and additionally NPV could reduce. However, for this meta-analysis there was no correlation between FNR and axillary pCR ($P=0.714$).

The sentinel lymph node procedure for axillary staging seems inappropriate in patients with clinically nodal stage 3. Our results showed significantly higher FNR in these cases (21.4 versus 13.1%, $P=0.049$). Periclavicular malignant lymph nodes are not always associated with presence of axillary lymph node metastases.^{31,32} Consequently, FNR can increase since metastases may be located elsewhere.

Noninvasive nodal restaging after neoadjuvant systemic therapy in clinically node positive patients with breast cancer prior to sentinel lymph node biopsy could be of additional value.³³ In our meta-analysis, two studies included only pathologically confirmed node positive patients who converted to clinically node negative after neoadjuvant systemic therapy.^{17,20} In these studies physical examination, ultrasound or MRI was used for nodal restaging. Nonetheless the pooled FNR for these two studies was not lower than the pooled FNR in the other studies (14.7 versus 15.2%, $P=0.885$). For these two studies it might be possible that

many true positive patients were excluded disproportionate to few false negative patients.

One of the inclusion criterion for this meta-analysis was cytological or histopathological confirmation of nodal malignancy at diagnosis, since node positivity based on clinical imaging and examination results in inaccurate nodal staging.³⁴⁻³⁶ Recently, Feng et al. demonstrated the importance of pathological confirmation for axillary nodal staging.³⁷ In their study, the PPV for axillary ultrasound in breast cancer patients improved from 79.6 towards 100%, when fine-needle cytology was added to the general axillary ultrasound. As a consequence, several studies were excluded for this meta-analysis due to differences in defining clinically node positive patients, like the SENTINA trial.³⁸⁻⁴⁰ In the SENTINA trial, pathologically confirmed nodal disease was not required for inclusion, resulting in the definition of node positive disease based on ultrasound. Including these studies for the meta-analysis may have led to overestimation of the results, as the axillary pCR rate is higher in these studies (40.0 versus 52.3%).^{14,38}

Our meta-analysis suggests that the sentinel node procedure after systemic treatment of a clinically node positive breast cancer patient is insufficiently accurate to identify the patient with axillary pCR. Other, rather noninvasive techniques for nodal restaging are warranted. In imaging, PET-CT^{41,42} and lymph node specific contrast-enhanced axillary MRI^{43,44} still need further exploration in order to prevent patients from invasive procedures for nodal staging, with reported PPV and NPV of 67 and 56% for PET-CT,⁴⁵ 75% and 43% for MRI, respectively.⁴⁶ Furthermore, marking malignant lymph nodes with radioactive iodine seeds is promising. Donker et al. achieved a FNR as low as 7% despite the fact that one third of the study patients had clinically nodal stage 3.⁴⁷ Yet the NPV of 83.3% is still insufficiently accurate to decide based on the MRI procedure that axillary surgery can be safely omitted.⁴⁸

As an alternative, a targeted axillary dissection can be performed. This procedure consists of a sentinel lymph node biopsy and removal of a clip containing previous positive node for the evaluation of pCR in clinically node positive breast cancer patients treated with neoadjuvant systemic therapy.⁴⁹ The clip-containing lymph node was not always in line with the sentinel node (1 out of 5 patients), indicating this technique might become a more appropriate tool for predicting pCR in future, as targeted axillary dissection could possibly identify more often the malignant node compared to the sentinel lymph node biopsy.

In conclusion, based on current evidence it seems not justified to omit further axillary treatment in every clinically node positive breast cancer patient with a negative sentinel lymph node biopsy after neoadjuvant systemic therapy.

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Appendix 2.1

Search strategy for systematic review and meta-analysis of sentinel lymph node biopsy in clinical node positive patients receiving neoadjuvant systemic therapy

Embase search strategy

1. breast.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
2. mamma.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
3. 1 or 2
4. cancer.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
5. carcinoma.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
6. neoplasmp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
7. malignancy.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
8. adenocarcinoma.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
9. 4 or 5 or 6 or 7 or 8
10. 3 and 9
11. sentinel lymph node biopsy.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
12. slnb.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]
13. sentinel node.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

14. 11 OR 12 OR 13

15. sensitivity.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

16. specificity.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

17. predictive value.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

18. positive predictive value.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

19. negative predictive value.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

20. likelihood ratio.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

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22. npv.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

23. diagnosis.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

24. 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23

25. axilla*.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

26. axilla.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

27. lymph.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

28. lymph*.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

29. 25 or 26 or 27 or 28

30. node.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

31. metastasis.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

32. nodes.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

33. 30 or 31 or 32

34. neoadjuvant.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

35. primary.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

36. preoperative.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

37. therapy.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

38. immunotherapy.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

39. chemotherapy.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

40. systemic.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

41. 34 or 35 or 36

42. 37 or 38 or 39 or 40

43. 41 and 42

44. 10 and 14 and 24 and 29 and 33 and 43

Pubmed search strategy

((((((("Breast Neoplasms"[Mesh]) OR ((((((carcinoma) OR neoplasm) OR neoplasms) OR malignancy) OR adenocarcinoma) OR cancer)))) AND (((breast OR mamma)) OR ((((((carcinoma) OR neoplasm) OR neoplasms) OR

malignancy) OR adenocarcinoma) OR cancer))) AND (((("Sentinel Lymph Node Biopsy"[Mesh]) OR sentinel lymph node biopsy) OR slnb)) AND (((((((sensitivity) OR specificity)) OR (((("Sensitivity and Specificity"[Mesh])) OR "Predictive Value of Tests"[Mesh])) OR negative predictive value) OR positive predictive value) OR likelihood ratio) OR diagnosis)) AND (((((((("Lymph Nodes"[Mesh]) OR "Lymphatic Vessels"[Mesh]) OR "Lymphatic Metastasis"[Mesh]) OR "Lymphatic System"[Mesh]) OR "Axilla"[Mesh]) OR axilla)) AND (((("Neoadjuvant Therapy"[Mesh]) OR neoadjuvant) OR preoperative) OR primary)) AND (((("Antineoplastic Agents"[Mesh]) OR chemotherapy) OR immunotherapy) OR systemic therapy)

Appendix 2.2

Results of bivariate meta-regression analyses

Identification rate

Covariates	Number of studies	Coefficient	P-value
Year of publication	7	-0.004	0.895
Study design	7	-0.027	0.369
Sample size	7	0.029	0.304
Clinical nodal stage	6	0.015	0.582

Meta-regression analyses for the outcome identification rate were obtained using covariates, which included year of publication (2013-2015 versus 2007-2012), study design (retrospective versus prospective study design), sample size of study population (less versus more than 100 patients), clinical nodal stage (clinical nodal stage 1-2 versus clinical nodal stage 1-3).

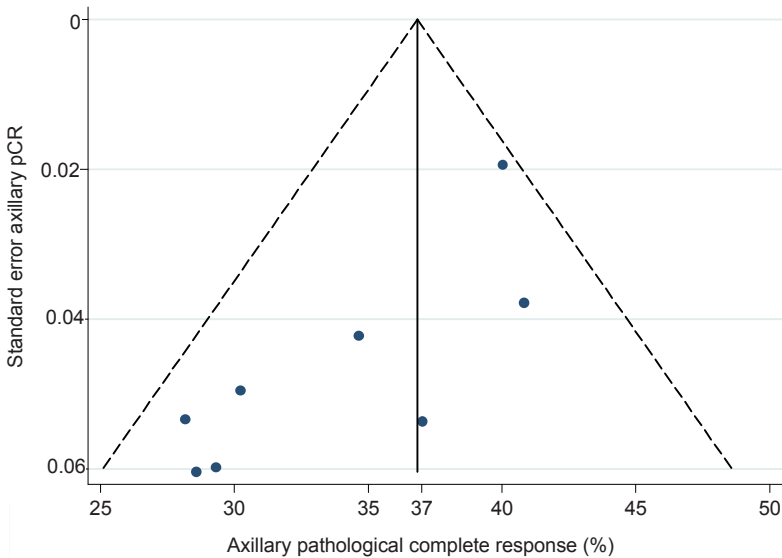
False-negative rate

Covariates	Number of studies	Coefficient	P-value
Year of publication	8	-0.042	0.306
Study design	8	-0.067	0.079
Sample size	8	-0.037	0.383
Clinical nodal stage	7	0.086	0.049
Definition isolated tumour cells	7	-0.019	0.678
Additional immunohistochemistry	8	-0.034	0.458

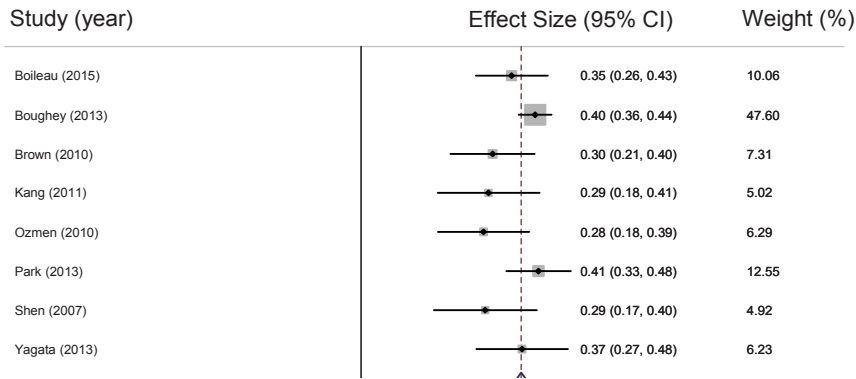
Meta-regression analyses for the outcome false-negative rate were obtained using covariates, which included year of publication (2013-2015 versus 2007-2012), study design (retrospective versus prospective study design), sample size of study population (less versus more than 100 patients), clinical nodal stage (clinical nodal stage 1-2 versus clinical nodal stage 1-3), definition of isolated tumour cells (isolated tumour cells considered as true negative versus isolated tumour cells considered as false negative), additional immunohistochemistry lymph nodes (no versus yes).

Appendix 2.3

a. Forest plot of axillary pathological complete response



3b. Funnel plot of axillary pathological complete response



Forest plot and funnel plot with pseudo 95% confidence intervals of axillary pathological complete response. The pooled axillary pCR was 36.8% ($I^2=41.5\%$, $P=0.102$).

Chapter 3

A novel less invasive approach for axillary staging after neoadjuvant chemotherapy in patients with axillary node positive breast cancer by combining Radioactive Iodine Seed localisation in the Axilla with the Sentinel node procedure (RISAS): a Dutch prospective multicentre validation study

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Abstract

Background

In one out of three patients with initial node positive (cN+) breast cancer neoadjuvant chemotherapy (NAC) results in an axillary pathologic complete response (ax-pCR). This urges the need for a less invasive axillary staging method. Recently introduced less invasive procedures are insufficient in accurately identifying ax-pCR. Therefore, we propose a novel less invasive axillary staging procedure, i.e. the RISAS procedure: Radioactive Iodine Seed localisation in the Axilla with the Sentinel node procedure, a combination of the MARI procedure and sentinel lymph node biopsy (SLNB).

Patients and methods

In this open single arm multicentre validation study 225 cN+ (biopsy proven) patients will undergo the RISAS procedure, in which a positive lymph node is marked by an I-125 seed prior to NAC. After completion of NAC, this I-125 seed marked lymph node is removed together with (additional) sentinel lymph node(s). The RISAS procedure is subsequently followed by a completion ALND. RISAS nodes will be compared to lymph nodes of the completion ALND specimen. Primary endpoint is accuracy of the RISAS procedure. Identification rate, false negative rate, negative predictive value and possible concordance between the MARI and sentinel lymph node(s) will be reported.

Conclusion

This prospective multicentre RISAS trial will enable us to validate the combination of MARI and SLNB for assessing axillary response to NAC in cN+ patients. If RISAS proves to be an accurate axillary staging procedure, ALND may safely be abandoned in case of ax-pCR confirmed by the RISAS procedure.

Introduction

Surgical management of the axilla in breast cancer evolved greatly in the past decades. In clinically node negative patients axillary staging shifted from axillary lymph node dissection (ALND) to sentinel lymph node biopsy (SLNB). In clinically node positive patients (cN+) ALND is still recommended for axillary staging.^{1,2} However, the shift from adjuvant to neoadjuvant chemotherapy (NAC) causes a growing need for a less invasive axillary staging approach, since NAC enables downstaging of axillary disease.^{3,4} In up to 36% of all patients, conversion of cN+ to axillary pathologic complete response (ax-pCR) as a result of NAC is reported.⁵ In case of HER2neu positive patients, ax-pCR is even achieved in up to 74% of patients when NAC is combined with HER2-targeted therapy.^{6,7} Patients in whom ax-pCR is achieved are not expected to benefit from ALND, but a significant proportion will suffer from side effects. Consequently, routine ALND is increasingly being regarded as overtreatment in these patients.

Until now, imaging techniques have not been able to accurately identify ax-pCR. As for surgical techniques, various less invasive axillary staging procedures have been proposed for cN+ patients after completion of NAC. The SLNB seems to be insufficient with reported false negative rates (FNRs) ranging from 7% to 25%.^{8,9} Since SLNB aims to identify patients with ax-pCR without missing residual disease, a negative predictive value (NPV) of 95% or higher is desirable. A high NPV is especially important since residual tumour is considered resistant to the administered chemotherapy and may require additional treatment. However, NPVs for SLNB do not exceed 86%,⁵ which means that residual axillary disease is missed in 1 out of every 6 patients in whom ax-pCR is suggested by the sentinel lymph node(s) (SLN(s)). Factors contributing to the improvement of FNR/NPV of SLNB are use of the dual tracer technique and removal of at least 3 SLNs.^{8,10-12} In daily practice, the latter is not always achievable, as was demonstrated by the SENTINA trial in which most patients had only 1 to 2 SLN(s) removed.¹² Donker et al. introduced an alternative, less invasive axillary staging procedure: the MARI procedure (Marking Axillary lymph nodes with Radioactive Iodine seeds).¹³ In this procedure, an I-125 seed is placed in the biopsy proven positive axillary lymph node prior to NAC. After completion of NAC, the I-125 seed-containing node is removed at time of breast surgery. In 98 pre-treatment cN+ patients an identification rate of 97% and a FNR of 7% was reported. MARI has a reported NPV of 83%, thus residual axillary disease is still overlooked to the same extent as with SLNB.^{13,14} In conclusion, both MARI and SLNB remain insufficiently accurate as solitary staging procedures.

Caudle et al. proposed Targeted Axillary Dissection (TAD),¹⁵ involving clip placement in a biopsy proven positive axillary lymph node prior to NAC followed by localisation of this clipped node with an I-125 seed after completion of NAC.

Excision of the I-125 seed marked node was combined with excision of SLN(s). All cN+ patients treated with NAC and in whom the biopsied node was marked with a clip were included in a prospective registry. Retrospectively, eighty-five patients that underwent TAD followed by a completion ALND were identified. A FNR of 2% and a NPV of 97% were reported.¹⁵ These results are promising; however, the study is hampered by a single centre study design and a small sample size with only 35 patients in whom ax-PCR was achieved. Consequently, widespread implementation of TAD is not recommended before a large, preferably multicentre, validation study is performed.

Therefore, we propose a multicentre, open, single arm validation study in which the RISAS procedure, which consists of Radioactive Iodine Seed localisation in the Axilla combined with the SLNB, is investigated. In order to validate RISAS, a completion ALND will be performed in all patients. The I-125 seed is placed prior to the start of NAC. If this study proves that RISAS accurately predicts axillary nodal status after completion of NAC, the ALND and its concomitant morbidity may safely be omitted and prevented in patients with ax-pCR predicted by RISAS.

Patients and methods

The RISAS study is an open single arm non-inferiority multicentre validation study to assess accuracy of RISAS for axillary staging after NAC in pre-treatment cN+ breast cancer patients. Nodal positivity is always confirmed by pathological assessment before start of NAC in case of suspicious nodes on axillary ultrasound. Patients will be recruited in approximately eight participating hospitals in The Netherlands over a period of two years. Each hospital has at least one year of experience with I-125 seed-guided localisation. Given a non-inferiority margin of 6.25% and assuming a prevalence of a positive ALND of 64%, a total of 225 patients is needed in order to test non-inferiority of RISAS compared with ALND. Patients fulfilling all eligibility criteria will – after completion of NAC – undergo the RISAS procedure and subsequent completion ALND (Table 3.1). Patients in whom I-125 seed placement is not possible and in whom the RISAS procedure is not followed by completion ALND will be excluded from final analysis. Chemotherapy regimens will be determined according to current Dutch breast cancer guidelines¹ and multidisciplinary team decision making. In HER2neu positive patients NAC is combined with HER2-targeted therapy.

The RISAS procedure consists of the MARI-procedure combined with SLNB. First, the most suspicious node is sampled by means of core needle biopsy or fine needle aspiration in order to confirm nodal positivity. Subsequently, the MARI-procedure is performed. This consists of marking the pathologically proven

positive lymph node with an I-125 seed prior to the start of NAC under ultrasound guidance. Retrieval of the sampled node is facilitated by reporting multiple characteristics of this node, e.g. diameter of the node and diameter of the cortex. Furthermore, the radiologist will capture an image of the biopsied lymph node. Iodine seeds with a maximum activity of 7.4 MBq (0.2 mCi) are used.¹⁶ SLNB will be performed according to the standard dual tracer technique. After excision of the RISAS node(s), the surgeon will proceed with the completion ALND. The RISAS node(s) and the remaining nodes of the axillary basin will be secured separately.

Table 3.1 Eligibility criteria.

Inclusion criteria	Exclusion criteria
Female, age \geq 18 years	Pregnancy or nursing
Invasive breast cancer with biopsy proven positive axillary lymph node(s)	Contraindications to RISAS (e.g. allergic reaction to blue dye)
NAC	Recurrent breast cancer
	History of axillary surgery or radiotherapy
	Positive infra- and / or supraclavicular lymph nodes
	Distant metastasis

Abbreviations: *NAC* neoadjuvant chemotherapy, *RISAS* Radioactive Iodine Seed localisation in the Axilla combined with the Sentinel node procedure.

The pathologist will assess the RISAS nodes and remaining nodes of the completion ALND specimen separately. Presence of isolated tumour cells, micrometastases and / or macrometastases will be registered. Routine H&E staining will be performed on all nodes. Immunohistochemistry will only be performed on each RISAS node, which is negative on H&E staining, irrespective of ALND specimen findings.

This study has been approved by the Institutional Review Board of the Erasmus Medical Centre (MEC-2016-412) and registered at ClinicalTrials.gov (NCT02800317).

Basic statistical analysis will be used. The test outcome of both RISAS and completion ALND can be either negative or positive. If the test outcome of RISAS is positive, the test outcome of the completion ALND will also be considered positive. The outcomes will be formulated in a 2x2 contingency table. The primary endpoint of our study is accuracy of the RISAS procedure and will be defined by calculating sensitivity, NPV and FNR.

Discussion

With the growing need for less invasive axillary staging after NAC, various procedures have been proposed. MARI and SLNB remain insufficient for this purpose since they miss potentially therapy resistant disease to a rather high extent.^{5,11,13} TAD shows promising results, but due to the retrospective analysis of patients and small sample size of this single centre study it seems premature to implement this procedure as standard practice and replace ALND.¹⁵ Our present RISAS study will provide answers to questions regarding TAD, the role of immunohistochemistry and what to do if fewer than two nodes will be removed.¹⁷ An overview of the studies proposing different less invasive axillary staging procedures is provided in Table 3.2.

Table 3.2 Overview of studies comparing less invasive surgical procedures with axillary lymph node dissection for axillary staging after NAC in pre-treatment cN+ patients.

Type of procedure	ACOSOG Z1071 ¹¹ SLNB	MARI-procedure ¹³ MARI	TAD ¹⁵ SLNB + I-125 seed placement in the clipped node after completion of NAC	RISAS SLNB + I-125 seed placement prior to NAC
Sample size*	687	98	85	225
Prevalence axillary ax-pCR	40.0	26.0	41.0	#
Identification rate (%)	92.7	97.0	100	#
FNR (%)	14.7	7.0	2.0	#
NPV (%)	82.0	83.0	97.2	#

* This includes all patients in whom concomitant ALND is performed, # pending on results.

Abbreviations: *SLNB* sentinel lymph node biopsy, *MARI* Marking the Axillary lymph node with Radioactive Iodine seeds, *TAD* Targeted Axillary Dissection, *RISAS* Radioactive Iodine seed localisation in the Axilla combined with Sentinel node procedure, *I-125 seed* iodine-125 seed, *NAC* neoadjuvant chemotherapy, *ax-pCR* pathologic complete response, *FNR* false-negative rate, *NPV* negative predictive value.

Since RISAS combines MARI and SLNB, the accuracy of RISAS is supposed not to be hindered by shortcomings of the solitary techniques. Metastatic lymph nodes may block and deviate normal lymphatic drainage, resulting in “false SLN(s)”. Given the fact that the RISAS procedure not only retrieves (false) SLNs but also pre-treatment pathologically proven metastatic lymph nodes it has the potential to be equal to ALND in staging of the axilla. Caudle et al. already demonstrated that the clipped node was not retrieved as an SLN in 23% of patients.¹⁵

An advantage of RISAS compared to TAD is that the biopsy proven positive node is primarily marked by an I-125 seed and not preceded by clip placement. Exact placement of the I-125 seed directly adjacent to the clip may be challenging, thus possibly hampering accuracy. Furthermore, direct marking of the lymph node by I-125 seed obviates an extra hospital visit after completion of NAC, which

minimizes patient burden, and it obviates an additional procedure, which reduces logistic issues.

Conclusion

The RISAS study is a multicentre prospective non-inferiority study to validate the combination of MARI and SN for predicting axillary response to NAC in pre-treatment cN+ patients. The ultimate goal is to identify ax-pCR patients by a minimally invasive procedure, in order to prevent unnecessary ALND with its associated side effects without comprising oncologic safety. ALND may then be rendered as management procedure only for patients with residual axillary disease.

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Part II

Non-invasive lymph node staging

Chapter 4

Routine use of standard breast MRI compared to axillary ultrasound for differentiating between no, limited and advanced axillary nodal disease in newly diagnosed breast cancer patients

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ML Smidt, MBI Lobbes

Eur J Radiol 2016;85:2288-2294

Abstract

Objectives

To compare standard breast MRI to dedicated axillary ultrasound (with or without tissue sampling) for differentiating between no, limited and advanced axillary nodal disease in breast cancer patients.

Methods

All patients who underwent breast MRI and dedicated axillary ultrasound between 2009 and 2014 were eligible. Exclusion criteria were recurrent disease, neoadjuvant systemic therapy and not receiving completion axillary lymph node dissection after positive sentinel lymph node biopsy (SLNB). Two radiologists independently reassessed all MRI exams. Axillary ultrasound findings were retrospectively collected. Probability of advanced axillary nodal disease (pN2-3) given clinically node negative (cNo) or limited (cN1) findings was calculated, with corresponding negative predictive value (NPV) to exclude pN2-3 and positive predictive value (PPV) to identify axillary nodal disease. Histopathology served as gold standard.

Results

A total of 377 cases resulted in 81.4% no, 14.4% limited and 4.2% advanced axillary nodal disease at final histopathology. Probability of pN2-3 given cNo for breast MRI and axillary ultrasound was 0.7–0.9% versus 1.5% and probability of pN2-3 given cN1 was 11.6–15.4% versus 29.0%. When cN1 on breast MRI was observed, PPV to identify positive axillary nodal disease was 50.7% and 59.0%.

Conclusions

Evaluation of axillary nodal status on standard breast MRI is comparable to dedicated axillary ultrasound in breast cancer patients. In patients who underwent preoperative standard breast MRI, axillary ultrasound is only required in case of suspicious nodal findings on MRI.

Introduction

Nowadays, preoperative axillary nodal staging in breast cancer patients consists of axillary ultrasound with concomitant tissue sampling if indicated.¹ Although, detection of every positive node was important in the past,² recent studies such as the ACOSOG Z0011, IBCSG 23-01 and AATRM 048/13/2000 trials have shown that completion axillary lymph node dissection (ALND) after the detection of limited axillary nodal disease (i.e. 1–3 positive nodes) does not improve prognosis.^{3–5} As a consequence, excluding advanced axillary nodal disease (i.e. more than 3 positive nodes) rather than detecting clinically node positive disease is becoming increasingly important.

Previous studies addressing the diagnostic performance of axillary ultrasound reported a negative predictive value (NPV) of 96% to exclude advanced nodal disease in case of node negative findings.^{6,7} However, axillary ultrasound appears unable to differentiate between limited and advanced axillary nodal disease in case of positive axillary ultrasound findings, with a reported NPV of 50%.⁸

According to a recent systematic review, MRI might be a promising non-invasive nodal staging tool for preoperative evaluation of the axilla to determine node negative and node positive disease in breast cancer patients.⁹ However, the publications studied in this review did not investigate the ability of MRI to determine the number of positive nodes for differentiating between no, limited and advanced axillary nodal disease. Only a few studies compared MRI to ultrasonography, the most frequently used imaging modality for this purpose in daily practice.¹⁰ If preoperative MRI, which is often part of the diagnostic work-up of newly diagnosed breast cancer patients, could improve nodal staging, axillary treatment could be even more patient-tailored. Thus, the aim of this study was to investigate the diagnostic performance of standard breast MRI compared to dedicated axillary ultrasound to differentiate between no, limited and advanced axillary nodal disease, with pathology serving as the gold standard.

Materials and methods

Patient selection

All patients diagnosed between 2009 and 2014 with invasive breast cancer who underwent both standard breast MRI and axillary ultrasound prior to surgery were eligible for this study. Exclusion criteria were recurrent breast cancer, patients primarily treated with neoadjuvant systemic therapy or patients with a positive sentinel lymph node biopsy (SLNB) who did not receive a completion ALND. Due to the retrospective design of this study, the necessity to acquire informed

consent from the study subjects was waived by the local medical ethics committee.

Standard breast MRI exam and image analysis

All included patients underwent a standard breast MRI protocol to evaluate breast cancer extent and the presence of contralateral breast cancer. Breast MRI was performed in prone position on two different 1.5 T MRI scanners (Ingenia and Intera, Philips Healthcare, Best, the Netherlands). Both MRI scanners used a body coil, which was in 2011 replaced by a dedicated 16-channel breast coil. Evaluation of the axillary lymph nodes was performed on a non-enhanced 3D T2W Turbo Spin Echo sequence. This sequence is part of the standard breast MRI protocol, which further consisted of dynamic, contrast-enhanced T1W sequence protocols using fat saturation and diffusion weighted imaging (DWI). Over the years, the MR sequence protocols were only slightly changed (Appendix 4.1).

A resident in radiology (M.S.) with two years of experience in breast radiology, pre-screened all breast MRI exams on technical quality criteria. Eligibility criteria were inclusion of the full axillary region including for example the sternoclavicular joint, axillary vein and the latissimus dorsi muscle. In addition, the axillary region had to be free of motion artefacts or inadequate signal-to-noise-ratio.

All axillary lymph nodes (i.e. axillary level 1–3, without assessment of the periclavicular and/or internal mammary lymph nodes) within one patient were separately counted and qualitatively assessed by two expert breast radiologists with 7 years (M.B.I.L.) and 5 years (S.V.) of breast imaging experience, respectively. Both radiologists were blinded for each other's results and to the histopathological outcome of the tumour and lymph nodes. However, radiologists were aware of the clinical tumour size, as assessed on mammography, ultrasound and/or breast MRI, similar to clinical practice.

Each individual lymph node was scored according to the criteria previously defined by Baltzer et al., using a confidence scale from 0 (no lymph nodes) to 4 (definitely malignant).¹¹ Suspicious characteristics included irregular margins, inhomogeneous cortex, perifocal edema, absent fatty hilum, asymmetry, and absence of chemical shift artefacts (Figure 4.1).^{11–13}

Clinical axillary nodal staging was based on the number of suspicious axillary lymph nodes: none (cNo), limited (cN1, 1–3 suspicious lymph nodes) and advanced (cN2–3, >3 suspicious lymph nodes) axillary nodal disease. In patients diagnosed with bilateral invasive breast cancer, lymph nodes were assessed in both axillae separately.

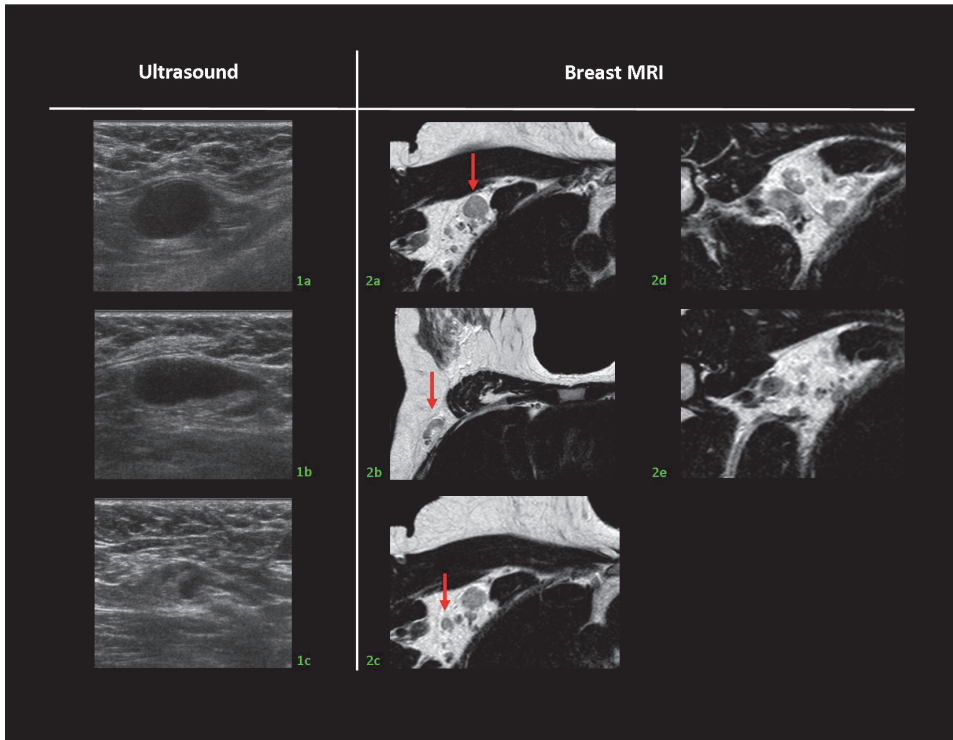


Figure 4.1 Transversal T2-weighted images of right axillary region of a female patient with a 45 mm large invasive ductal carcinoma in her right breast. Dedicated axillary ultrasound reported three suspicious axillary nodes (cN₁), whereas on MRI the radiologists predicted respectively 10 and 7 suspicious axillary lymph nodes (both cN₂₋₃). The red arrows on MR Images 2a–c represent the corresponding suspicious nodes detected during axillary ultrasound on 1a–c. The MR Images 2d and 2e demonstrate additional suspicious axillary lymph nodes. Histopathology of the axillary lymph node dissection reported a total of 9 axillary lymph node metastases (pN₂₋₃).

Axillary ultrasound

Axillary ultrasound exams were performed by dedicated breast radiologists, using an ATL-HDI5000 system in combination with a linear 5 to 12-MHz array transducer, which in 2011 was replaced by an iU22-xMATRIX ultrasound system with a linear 2 to 17-MHz array transducer (both systems: Philips Healthcare, Best, the Netherlands). Criteria for a suspicious axillary lymph node on axillary ultrasound consisted of diffuse cortical thickening, focal cortical mass and/or thickening and loss of the fatty hilum.⁶ The result of these exams, including the number of suspicious axillary lymph nodes, was extracted from the radiology report for each individual patient. Tissue sampling was performed in case of suspicious axillary lymph nodes using 16–18 gauge core needle biopsy. When core

needle biopsy was challenging, fine needle aspiration cytology was used. In cases of multiple suspicious nodes, only the most suspicious node was sampled and the number of suspicious nodes was reported.^{8,14} In this study, dedicated axillary ultrasound was defined as axillary ultrasound with or without tissue sampling if deemed necessary by the radiologist on call.

In contrast to MRI, clinically node positive disease with ultrasound was always histopathologically confirmed. Diagnostic performance of axillary ultrasound was described earlier by Schipper et al., by using the same study subjects between 2009 and 2012 (n=243).⁸ In our current study, axillary ultrasound results were used for per-patient comparison with results of breast MRI.

Surgical nodal staging

In clinically node negative patients, based on negative axillary ultrasound findings or sampled tissue without evidence of tumour cells, SLNB was performed. The sentinel lymph node was identified by using a triple technique consisting of lymphoscintigraphy (using 80 MBq Technetium-99 m nanocolloid injected peri-areolar), blue dye to detect lymphatic vessels (Bleu Patente V[®]; Guerbet, Aulnay-sous-Bois, France), and a gamma probe to detect radioactivity. In case of one or more histopathologically confirmed metastases, a completion level I-II ALND was performed. In clinically node positive patients, based on tissue sampling with evidence of tumour cells after suspicious axillary ultrasound findings, ALND was performed.

Histopathological evaluation

Sentinel lymph nodes were sliced with a maximum thickness of 3 mm and paraffin embedded for histological evaluation. Step-sectioning at 500 micrometer intervals was done at three levels and were stained with haematoxylin and eosin (H&E). If conventional H&E slices were negative, a mouse anti-human MNF116 antibody (Dako, Glostrup, Denmark) was used for cytokeratin immunohistochemical staining.

All lymph nodes harvested by axillary lymph node dissection were paraffin embedded after 24 h of fixation for histological evaluation. Lymph nodes larger than 5 mm were sliced at a maximum thickness of 3 mm, and stained with H&E. Each node was recorded as either benign or as isolated tumour cells (≤ 0.2 mm), micrometastasis ($0.2 \leq 2.0$ mm) or macrometastasis (>2.0 mm).¹⁵ In this study, isolated tumour cells and micrometastases were considered negative. Pathological axillary nodal staging was based on the number of malignant axillary lymph nodes: none (pNo), limited (pN1, 1–3 lymph node metastases) and advanced (pN2–3, >3 lymph node metastases) axillary nodal disease.

Statistical analysis

Statistical analyses were performed by using Statistical Package for the Social Sciences software (version 22, IBM, Armonk, New York, USA). The probability of presence of advanced axillary nodal disease (pN2-3) in case of test results cNo or cN1, was calculated as $pN2-3 / (pNo + pN1 + pN2-3)$ for respectively cNo and cN1. In case of test result cN2-3, the probability of correctly predicted pN2-3 was calculated as $pN2-3 / cN2-3$. Discrepant cases between MRI and axillary ultrasound were described, to investigate whether results of both techniques could further decrease the probability of pN2-3 given cNo or cN1. A subgroup analysis between patients who underwent standard breast MRI between 2009 and 2010 (using a body coil) and 2011–2014 (using a dedicated breast coil) was performed to investigate whether there were any differences between both subgroups.

Interobserver agreement between the two readers reviewing the breast MRI exams, according to the initial confidence scale 0–4, was calculated by quadratic weighted kappa coefficient (κ) to determine the agreement between both readers for the evaluation of axillary nodal staging on standard breast MRI.¹⁶ A score of 0–2 was considered benign, a score of 3–4 malignant.

Results

Between 2009 and 2014, 803 patients were diagnosed with invasive breast cancer and underwent standard breast MRI and axillary ultrasound. After screening all MRI exams, a total of 377 MRI exams (60.6% of all eligible MRI exams) in 373 patients were included, due to bilateral breast cancer in four patients (Figure 4.2). Patient and tumour characteristics are shown in detail in Table 4.1.

Final pathologic report showed pNo in 81.4%, pN1 in 14.4% and pN2-3 in 4.2% of the patients (Table 4.2). Clinical nodal status on MRI and ultrasound with corresponding pathological nodal status is shown in Table 4.2. Interobserver agreement between the radiologists on standard breast MRI was considered substantial ($\kappa=0.68$, 95% CI 0.58–0.77).

Negative axillary findings (cNo)

With ultrasound, 344 cases were staged as cNo, of which 307 were pNo (89.2%), 32 were pN1 (10.0%) and 5 were pN2-3 (1.5%). Thus, probability of pN2-3 given cNo was 1.5%, with a corresponding NPV of 98.5% (95% CI 96.4–99.5%) to exclude advanced axillary nodal disease in case of cNo (Table 4.3).

According to reader 1, a total of 321 breast MRI exams were staged as cNo, of which 286 were pNo (89.1%), 32 were pN1 (10.0%) and 3 were pN2-3 (0.9%). Thus, probability of pN2-3 given cNo was 0.9%, with a corresponding NPV of 99.1%

(95% CI 97.1–99.8%) to exclude advanced axillary nodal disease in case of cNo. In case of cNo according to reader 1 on MRI with pN2-3 as histopathological result (n=3), axillary ultrasound predicted 2 cases as cN1 and one as cNo.

Reader 2 staged a total of 299 breast MRI exams as cNo, of which 273 were pNo (91.3%), 24 were pN1 (8.0%) and 2 were pN2-3 (0.7%). Probability of pN2-3 given cNo was 0.7%, with a corresponding NPV of 99.3% (95% CI 97.3–99.9%) to exclude advanced axillary nodal disease in case of cNo. In case of cNo according to reader 2 on MRI with pN2-3 as histopathological result (n = 2), axillary ultrasound predicted one case as cN1 and one cNo.

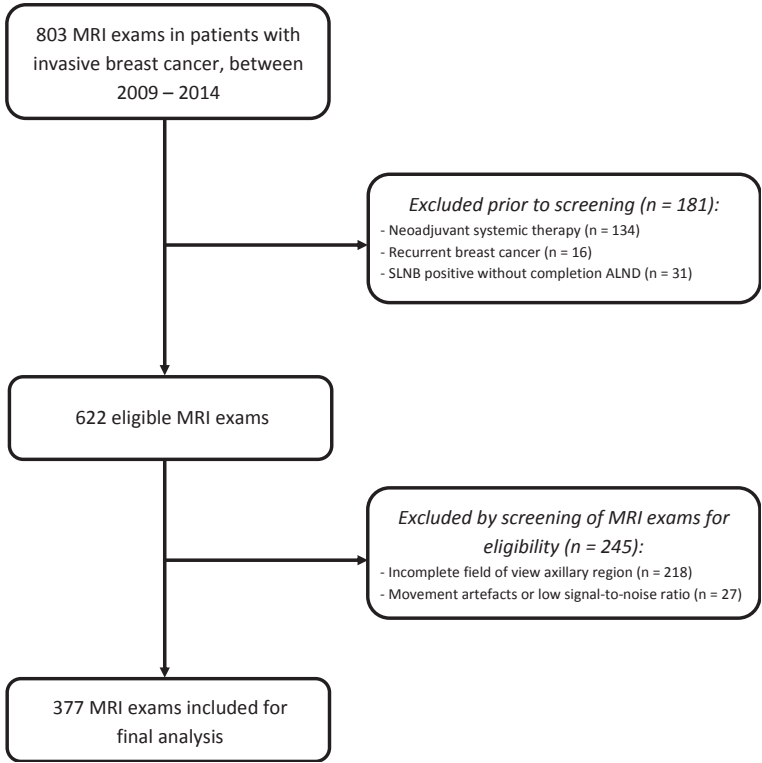


Figure 4.2 Flowchart of patient inclusion. Abbreviations: *SLNB* sentinel lymph node biopsy, *ALND* axillary lymph node dissection.

Table 4.1 General patient and tumour characteristics.

Number of patients	377
Mean age (y) (range)	58 (21-81)
Mean clinical tumour size (mm) (range)	25.0 (2-100)
cT stage (%)	
T ₁	209 (55.4)
T ₂	133 (35.3)
T ₃	35 (9.3)
Uni- and multifocal tumours (%)	
Unifocal	314 (83.3)
Multifocal	63 (16.7)
Tumour type (%)	
Ductal	311 (82.5)
Lobular	42 (11.1)
Mixed ductal and lobular	10 (2.7)
Other [#]	14 (3.7)
Tumour grade (%)	
1	97 (25.7)
2	173 (45.9)
3	104 (27.6)
Missing	3 (0.8)
pT stage (%)	
Tis	5 (1.3)
T ₁	268 (71.1)
T ₂	89 (23.6)
T ₃	13 (3.5)
T ₄	2 (0.5)
Hormonal and receptor status (%)	
ER/PR positive, Her2 negative	284 (75.3)
ER/PR negative, Her2 positive	16 (4.2)
Triple negative	39 (10.4)
Triple positive	35 (9.3)
Not determined	3 (0.8)
Axillary surgery (%)	
SLNB	292 (77.5)
SLNB with completion ALND	49 (13.0)
ALND	36 (9.5)

Abbreviations: *cT stage* clinical tumour stage, *pT stage* pathological tumour stage, *pN stage* pathological nodal stage, *SLNB* sentinel lymph node biopsy, *ALND* axillary lymph node dissection. # Other tumour types: ductal carcinoma in situ, mucinous carcinoma, adenoid cystic carcinoma and papillary carcinoma.

Limited axillary findings (cN₁)

According to ultrasound, 31 cases were staged as cN₁, of which 22 were pN₁ (71.0%) and 9 were pN₂₋₃ (29.0%). Thus, underestimation of pN₂₋₃ given cN₁ was 29.0%, with a corresponding NPV of 71.0% (95% CI 51.8–85.1%) to exclude advanced axillary nodal disease in case of cN₁ (Table 4.3).

According to reader 1, a total of 39 breast MRI exams were staged as cN₁, of which 16 were pNo (41.0%), 17 were pN₁ (43.6%) and 6 were pN₂₋₃ (15.4%). Thus,

PPV to identify positive axillary nodal disease was 59.0% (95% CI 42.2–74.0%) and probability of pN2-3 given cN1 was 15.4%, resulting in a corresponding NPV of 84.6% (95% CI 68.8–93.6%) to exclude advanced axillary nodal disease in case of cN1. In case of cN1 according to reader 1 on MRI with pN2-3 as histopathological result (n=6), axillary ultrasound predicted three cases as cN1 and three cases as cNo.

Reader 2 staged 69 breast MRI exams as cN1, of which 34 were pNo (49.3%), 27 were pN1 (39.1%) and 8 were pN2-3 (11.6%). PPV to identify positive axillary nodal disease was 50.7% (95% CI 38.5–62.9%) and probability of pN2-3 given cN1 was 11.6%, resulting in a NPV of 88.4% (95% CI 77.9–94.5%) to exclude advanced axillary nodal disease in case of cN1. In case of cN1 according to reader 2 on MRI with pN2-3 as histopathological result (n=8), axillary ultrasound predicted five cases as cN1 and three cases as cNo.

Table 4.2 Clinical nodal stage according to ultrasound- and MRI findings, with corresponding pathological nodal stage.

Axillary pN stage	pNo (n=241)	pNoi+ (n=35)	pNi(mi) (n=31)	pN1 (n=54)	pN2-3 (n=16)	Total (n=377)
MRI reader 1						
cNo	226	34	26	32	3	321
cN1	11	1	4	17	6	39
cN2-3	4	-	1	5	7	17
MRI reader 2						
cNo	216	30	27	24	2	299
cN1	25	5	4	27	8	69
cN2-3	-	-	-	3	6	9
Ultrasound						
cNo	241	35	31	32	5	344
cN1	-	-	-	22	9	31
cN2-3	-	-	-	-	2	2

Abbreviations: cN clinical nodal stage, pN pathological nodal stage, pNoi+ isolated tumour cells, pNi(mi) micrometastases

Table 4.3 Probability of pN2-3 given cNo, cN1 or cN2-3, with corresponding NPV to exclude advanced axillary nodal disease and PPV to identify axillary nodal disease.

	MRI reader 1	MRI reader 2	Ultrasound
cNo	n=321	n=299	n=344
Underestimation pN2-3	0.9% (3/321)	0.7% (2/299)	1.5% (5/344)
NPV	99.1% (318/321)	99.3% (297/299)	98.5% (339/344)
cN1	n=39	n=69	n=31
Underestimation pN2-3	15.4% (6/39)	11.6% (8/69)	29.0% (9/31)
NPV	84.6% (33/39)	88.4% (61/69)	71.0% (22/31)
PPV	59.0% (23/39)	50.7% (35/69)	100% (31/31)
cN2-3	n=17	n=9	n=2
Correctly identified pN2-3	41.2% (7/17)	66.7% (6/9)	100% (2/2)

Abbreviations: cN clinical nodal stage, NPV negative predictive value, PPV positive predictive value.

Advanced axillary findings (cN2-3)

According to breast MRI, reader 1 and reader 2 correctly predicted 7 (41.2%) and 6 (66.7%) cases with pN2-3 out of 17 and 9 patients as cN2-3 according to MRI, whereas ultrasound predicted 2 (100%) cases with pN2-3 out of 2 patients as cN2-3 according to ultrasound (Table 4.3).

Comparison MR protocols 2009–2010 and 2011–2014

In November 2010, the MR protocols for standard breast MRI changed (Appendix 4.1). In this study, 127 MRI exams were performed between 2009 and 2010 and 250 between 2011 and 2014. In case of cNo, this resulted in the following probability of pN2-3 given cNo: 0.9% versus 0.9% for reader 1 and 1.0% versus 0.5% for reader 2. In case of cN1, this resulted in the following probability of pN2-3 given cN1: 14.3% versus 16.0% for reader 1 and 13.6% versus 10.6% for reader 2.

Discussion

As axillary treatment based on clinical nodal status is becoming important, accurate assessment of the number of axillary nodal metastases is mandatory. Recent studies demonstrated axillary ultrasound is insufficiently accurate for differentiating between cN1 and cN2-3, while other studies suggest that MRI could be of additional value for proper axillary nodal staging.^{9,17} Studies concerning axillary nodal staging with MRI were performed differentiating between axillary node negative and positive disease, instead of no, limited and advanced axillary nodal disease. We showed that axillary nodal staging on standard breast MRI is comparable to axillary ultrasound.

The importance of differentiating between limited and advanced axillary nodal disease, is based on its therapeutic consequences: locoregional radiation therapy. It has been known that the addition of locoregional radiation therapy to chemotherapy improves the prognosis of patients with advanced axillary nodal disease.^{18,19} However, the omission of additional treatment (completion ALND) in cNo patients with limited axillary nodal disease at final pathology, does not seem to affect prognosis.^{3–5,14} As a consequence, differentiation between limited and advanced axillary nodal disease is becoming clinically more relevant in order to identify patients who benefit from additional axillary treatment, since both surgical and radiation therapy cause side effects as well.²⁰

According to the current guideline of the European Society of Medical Oncology for primary breast cancer, axillary ultrasound is recommended for the assessment of regional lymph nodes.¹⁸ Previous studies concerning axillary ultrasound in breast cancer patients reported an inability to differentiate between

limited and advanced axillary nodal disease, because in 50–68% of the cN1 patients detected with ultrasound pathologically confirmed advanced axillary nodal disease was still present.^{8,17} Yet, a recent study by Verheuve et al. demonstrated a worse prognosis in node positive patients detected by ultrasound compared to SLNB-positive patients, indicating that preoperative axillary ultrasound remains important.²¹ In short, axillary ultrasound cannot differentiate between no, limited and advanced axillary nodal disease accurately, but it might be of benefit to differentiate between prognostic favourable and unfavourable patients.

Since many breast cancer patients undergo preoperative breast MRI to assess the extent of the disease in the breast, we studied whether lymph node staging would be more accurate using this imaging exam compared to axillary ultrasound. The added benefit of using standard breast MRI would be that no extra exams, such as dedicated axillary MR imaging, would be necessary. In this study, the non-enhanced T2 weighted sequence was chosen for axillary nodal staging, due to better anatomical characterization of the axillary lymph nodes compared to dynamic contrast-enhanced sequence. This is supported by a systematic review of Kuijs et al. who demonstrated that non-enhanced T2W sequence was one of the most accurate sequence protocols for axillary lymph node imaging in breast cancer patients, however the included studies did only differentiate between node negative and positive findings instead of differentiating between limited and advanced axillary nodal disease in addition.⁹

Recently, Hyun et al. performed a retrospective study to evaluate the use of breast MRI to differentiate between no, limited and advanced axillary disease. They showed similar percentages of probability of pN2-3 given cNo and cN1 in a cohort of 310 patients treated with primary surgery: 0.4% for cNo and 15.6% for cN1, compared to 0.7–0.9% and 11.6–15.6% in our study.²² In our opinion, this study had an important limitation, since it did not directly compare breast MRI results with the results of axillary ultrasound in a per-patient analysis, thereby missing important information to compare the results of the proposed new test (breast MRI) with the standard reference test (axillary ultrasound). Our results demonstrated a decrease in probability of pN2-3 given cN1 from 29.0% to 11.6–15.4%, when axillary ultrasound was replaced by breast MRI. Yet, breast MRI resulted in a relatively low PPV of 50.7% and 59.0% to identify node positive disease in case cN1 was observed. Consequently, the benefit of MRI in cN1 patients is limited. Besides, in cases of cN1 predicted by standard breast MRI with pN2-3 as histopathological result, axillary ultrasound did not further decrease the probability of pN2-3, yet second-look axillary ultrasound after suspicious axillary lymph nodes on MRI can be recommended due to improved selection of cNo.²³ Second-look axillary ultrasound is performed different compared to the axillary ultrasound in our study, because of the information of suspicious nodes on MRI.

In cN2-3 patients, MRI correctly predicted more patients with pN2-3 compared to ultrasound, but because of the small sample size these results should be interpreted with caution.

This study had several limitations. First, predictive values (PPV and NPV) depend on prevalence of axillary nodal disease.²⁴ Therefore, results should be interpreted with the prevalence of pN1 and pN2-3 in mind, which consisted of 18.6% of the patients with macrometastases at final histopathology in our cohort.

Second, there is a small chance of verification bias, due to referral of patients to biopsy based on test results of axillary ultrasound. Axillary treatment in our study population depends on clinical nodal status according to the axillary ultrasound: in case of node negative findings patients will receive SLNB, in case of node positive findings patients will receive ALND. Previous studies reported false negative rates of SLNB up to 9.8%, indicating that patients with a negative SLNB still had positive lymph nodes in the completion ALND.²⁵ As a consequence, this might have affected our results, as some patients with a negative SLNB could have positive lymph nodes in the remaining axillary lymph nodes.

Third, due to differences in prevalence of cNo, cN1 and cN2-3 between MRI (respectively for reader 1 and 2) and ultrasound, statistical analyses to compare these results together were not possible. As a consequence, MRI results of reader 1 and 2 could not be combined, as some cases were differently assessed by both readers. However, this study showed that MRI resulted in many false positive findings in case of cN1, indicating that MRI is not able to replace axillary ultrasound for preoperative nodal staging. In contrast to MRI, axillary ultrasound has no false positive results because of the histopathological results of the biopsy.

Fourth, many patients were excluded according to the applied exclusion criteria. These criteria included all patients treated with neoadjuvant systemic therapy, in which a higher amount of patients with advanced axillary nodal disease is likely to be expected. Furthermore, nearly 40% of all eligible MRI exams had to be excluded due to inadequate visualization of the axilla on standard breast MRI. The fact that these exams were used to assess breast cancer extent within the breast is the reason behind this high exclusion rate, as these scans were not primarily focussing on axillary imaging.

Finally, we used two different MRI scanners with different sequence protocols. In November 2010 the MR sequence protocol was slightly adjusted due to the introduction of a dedicated 16-channel breast coil (Appendix 4.1). During that time, slice thickness was reduced and in plane resolution slightly increased on both systems. Despite these modifications, we did not observe differences in probability of pN2-3 in case of cNo or cN1.

In conclusion, standard breast MRI in preoperative axillary lymph node staging is comparable to dedicated axillary ultrasound. In patients who underwent

preoperative standard breast MRI, axillary ultrasound is only required in case of suspicious nodal findings on MRI.

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Appendix 4.1

Overview MR protocols for standard breast MRI between 2009 – 2014

MRI 1: Philips Intera 1.5T.

	2009	2010	2011	2012	2013	2014
Coil	Sense body coil	Sense body coil	Sense breast 16-channel	Sense breast 16-channel	Sense breast 16-channel	Sense breast 16-channel
Matrix	512 x 512	512 x 512	384 x 384	384 x 384	384 x 384	384 x 384
Pixel spacing (mm)	0.742 ²	0.645 ²	0.959 ²	0.959 ²	0.959 ²	0.959 ²
Field of View (mm)	380 ²	330 ²	368 ²	368 ²	368 ²	368 ²
In-plane resolution (mm)	0.7 ²	0.6 ²	1.0 ²	1.0 ²	1.0 ²	1.0 ²
Repetition time (msec)	5922	3012	2000	2000	2000	2000
Echo time (msec)	110	110	204	205	205	258
Flip angle (degrees)	90	90	90	90	90	90
Echo train length	33	33	80	80	80	96
Slice thickness (mm)	3.0	3.0	2.0	2.0	2.0	2.0

MRI 2: Philips Ingenia 1.5T

	2009	2010	2011	2012	2013	2014
Coil	Sense body coil	Sense body coil	Sense breast 16-channel	Sense breast 16-channel	Sense breast 16-channel	Sense breast 16-channel
Matrix	512 x 512	512 x 512	400 x 400	400 x 400	400 x 400	400 x 400
Pixel spacing (mm)	0.684 ²	0.606 ²	0.854 ²	0.854 ²	0.854 ²	0.854 ²
Field of View (mm)	350 ²	310 ²	342 ²	342 ²	342 ²	342 ²
In-plane resolution (mm)	0.7 ²	0.6 ²	0.9 ²	0.9 ²	0.9 ²	0.9 ²
Repetition time (msec)	8213	6845	2000	2000	2000	2000
Echo time (msec)	110	110	215	218	218	218
Flip angle (°)	90	90	90	90	90	90
Echo train length	29	29	95	95	95	95
Slice thickness (mm)	3.0	3.0	2.0	2.0	2.0	2.0

Chapter 5

The diagnostic performance of gadofosveset-enhanced axillary MRI for nodal (re)staging in breast cancer patients: results of a validation study

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Abstract

Purpose

To evaluate diagnostic performance of gadofosveset-enhanced MRI (GDF-MRI) in addition to T2-weighted MRI (T2W-MRI) for nodal (re)staging in newly diagnosed breast cancer patients.

Methods

Ninety patients underwent axillary T2W- and GDF-MRI. Two radiologists independently scored each lymph node; first on T2W-MRI, subsequently adjusting their score on GDF-MRI. Diagnostic performance parameters were calculated on node-by-node and patient-by-patient validation with histopathology as the reference standard. Furthermore, learning curve analysis for reading GDF-MRI was performed.

Results

In patient-by-patient validation, overall reader 1 performance for T2W- and GDF-MRI was similar with area under the receiver operating characteristic curves (AUC) of 0.75 and 0.77 ($P=0.731$), while for reader 2 AUC-values were 0.79 and 0.72 ($P=0.156$). For node-by-node validation, AUC-values of T2W- and GDF-MRI were 0.76 and 0.82 ($P=0.018$) and 0.77 and 0.77 ($P=0.998$) for reader 1 and 2. The AUC for reader 1 of the first one-third nodes was 0.71, improving to 0.80 and 0.95 for the next and last one-third. Sensitivity, specificity, PPV and NPV improved from 38%, 89%, 56%, and 79% to 60%, 93%, 64% and 92%. AUC of reader 2 improved from 0.69 to 0.79.

Conclusion

The current study confirmed that GDF-MRI, in addition to T2W-MRI, has potential as non-invasive method for nodal (re)staging in breast cancer.

Introduction

Breast cancer is one of the most frequently diagnosed cancers among women, with a worldwide incidence rate of 1.7 million.¹ Survival rates have increased in the last decades due to improved diagnostic techniques and treatment regimens. Five year survival rates are up to 98% for early stage, lymph node negative breast cancer.² Consequently, more attention is afforded to maintaining quality of life by limiting overtreatment and its associated lifetime morbidity.

For a long time, axillary lymph node dissection (ALND) was routinely performed to assess nodal status in breast cancer patients. This procedure is associated with significant short- and long-term morbidity. Seroma, lymphedema, nerve injury and reduced shoulder function are reported in up to 49% of the patients after 3 years of follow-up.³⁻⁵ About 15 years ago, the sentinel lymph node biopsy (SLNB) became the standard procedure in clinically node negative patients. It was followed by a completion ALND in case of a positive sentinel lymph node(s).⁶ Nevertheless, SLNB remains an invasive procedure with short-term side effects in 25% of patients and long-term morbidity (for example lymphedema) in up to 6% of the patients.^{4,7,8}

As 74% of the sentinel lymph nodes show no metastasis, a non-invasive imaging technique able to identify these node negative patients would be a step forward in personalized treatment.⁹ It would result in a significant reduction of morbidity and increase the patient's quality of life, while maintaining the high survival rates achieved so far.

An accurate non-invasive nodal staging tool could also guide treatment in clinically node positive breast cancer patients. In cases of neoadjuvant systemic therapy, pathological complete response of axillary lymph node metastases is achieved in approximately 37%.¹⁰ Identifying these patients by a non-invasive imaging modality, and thereby avoiding an ALND, would further reduce breast cancer treatment related morbidity.

In rectal cancer, the use of magnetic resonance imaging (MRI) with a blood pool contrast agent, gadofosveset, improved diagnostic performance for lymph node staging.^{11,12} Consequently, a feasibility study of Schipper et al. on the use of gadofosveset-enhanced MRI for axillary nodal staging in ten breast cancer patients showed a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 86%, 94%, 75% and 97%, respectively. They concluded that gadofosveset-enhanced MRI is a promising tool to accurately detect node negative breast cancer patients.¹¹

The aim of this study was to prospectively assess the diagnostic performance of gadofosveset-enhanced MRI for nodal (re)staging in a larger population of newly diagnosed breast cancer patients using histopathology as reference standard.

Materials and methods

Setting and patients

The local medical ethics committee approved this prospective single centre study, which was performed from May 2012 until May 2016. Consecutive breast cancer patients were included after written informed consent was obtained. Inclusion criteria were patients with biopsy proven in situ or invasive breast cancer, scheduled for SLNB or ALND. Exclusion criteria were pregnancy, prior ipsilateral axillary surgery, a glomerular filtration rate <45 mL/min/1.73m², and contraindications for either MRI or gadofosveset. GDF-MRI was performed shortly before SLNB or ALND in standard setting. In case of neoadjuvant systemic therapy, axillary MRI was performed between the last course of systemic therapy and SLNB and/or ALND. This study was registered at ClinicalTrials.gov (NCT01609920).

Imaging

Axillary imaging was performed on a 3.0 Tesla MRI scanner (Achieva 3.0TX, Philips Healthcare, Best, The Netherlands) using a 32-channel cardiac coil with the patient in supine position and ipsilateral arm elevated. The imaging protocol consisted of the following parameters: First, a three-dimensional (3D) standard T2 weighted (T2W) turbo spin echo sequence without fat suppression was performed in 3 minutes 26 seconds (repetition time (TR) 2000 ms; echo time (TE) 153 ms; 90 degree flip angle; turbo spin echo factor 50; number of signal acquisitions (NSA) 1; slice thickness 2.50 mm; reconstruction section thickness 1.25 mm; field of view (FOV) 220 x 220 mm²; matrix 176 x 176; resulting in an in-plane resolution of 1.25 x 1.25 mm²).

Second, gadofosveset (Ablavar[®], Lantheus Medical Imaging, North Billerica, United States of America) contrast with a dose of 0.12 ml/kg bodyweight was intravenously administered, followed after 12 minutes by a 3D T1 weighted (T1W) gradient echo sequence in 5 minutes 7 seconds (TR 6.3 ms, TE 4.6 ms; 15 degree flip angle; NSA 2; slice thickness 1.25 mm; field-of-view 220 x 220 mm²; matrix size 176 x 176; resulting in an in-plane resolution of 1.25 x 1.25 mm²). The time interval of 12 minutes after contrast administration was chosen based on a previous publication.¹¹

Image evaluation

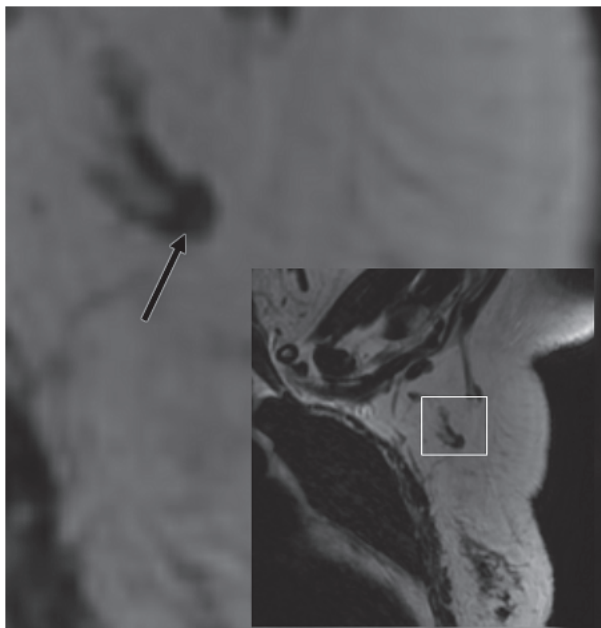
Two radiologists independently analyzed T2W-MRI and T2W-MRI combined with gadofosveset-enhanced MRI (GDF-MRI), blinded for histopathological findings and each other's results. Similar to clinical practice, radiologists were aware of

clinical tumour size, assessed on mammography, ultrasound and/or breast MRI. However, they were blinded for results of physical examination and axillary ultrasound. After each assessment on MRI, radiologists received direct feedback concerning final histopathology.

The first radiologist (R.B.T.) had 15 years of experience in pelvic MRI including eight years in GDF-MRI for lymph node assessment in rectal cancer, but no expertise in breast MRI [reader 1]. The second radiologist (M.L.) had seven years of experience in breast MRI and in axillary lymph node assessment using ultrasound and four years in axillary lymph node assessment on breast MRI, but no expertise in GDF-MRI [reader 2].

On T2W-MRI, each node was scored using a confidence level scale of 0 (definitely benign) to 4 (definitely malignant), using the criteria as described by Baltzer et al.¹² Suspicious characteristics were irregular margins, inhomogeneous cortex, perifocal edema and absent fatty hilum. Next, GDF-MRI was presented, and the radiologists were allowed to adjust their initial score. The GDF-MRI criteria were previously described by Lambregts et al.¹³; in short, a node was considered benign when it showed contrast hyperintensity after intravenous injection of gadofosveset. Malignant characteristics were absence of contrast hyperintensity and absence of an intact nodal border. Examples of imaging features of malignant lymph nodes on GDF-MRI are given in Figures 5.1 and 5.2.

A



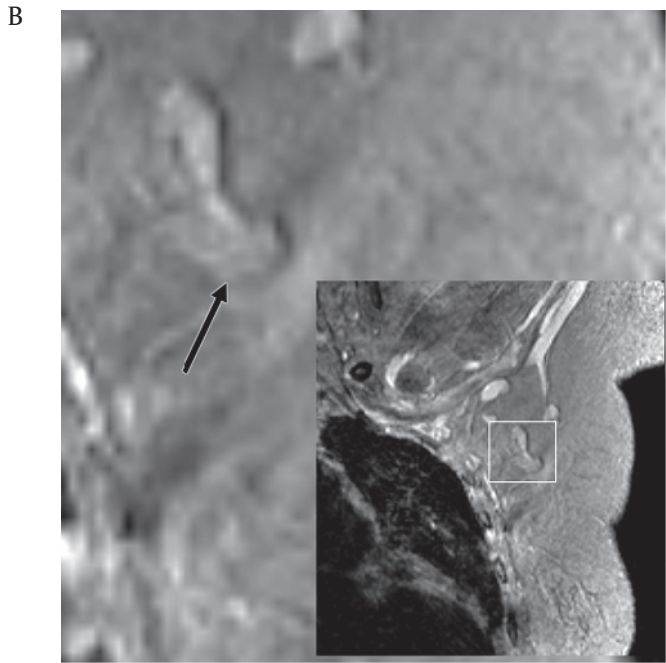


Figure 5.1 MR-images, after completion of neoadjuvant chemotherapy, of a 58-year old women with a 27 mm large invasive ductal carcinoma in her left breast, which was treated with neoadjuvant chemotherapy followed by lumpectomy with sentinel lymph node biopsy. (A) Coronal T2-weighted MR image demonstrates the presence of a fatty hilum in the lymph node. Therefore this lymph node was considered benign by both readers. (B) Coronal T1-weighted MR images after administration of gadofosveset (GDF-MRI) demonstrates the absence of an intact nodal border, which resulted in a malignant lymph node according to one of the two readers. Results of the corresponding histopathology at the sentinel lymph node biopsy revealed a 3.5 mm macrometastasis of this lymph node.

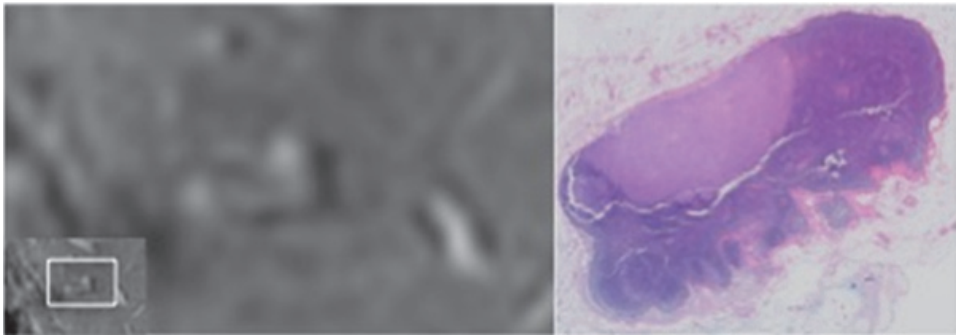


Figure 5.2 An example of a lymph node on coronal T1-weighted GDF-MRI. This node was scored as benign for both readers, based on the presence of a chemical shift artefact and a fatty hilum. However, as seen on the H&E coupe on the right, the dark area of the lymph node was not a fatty hilum, but was mimicked by a metastatic area.

Surgery

The sentinel lymph node was depicted on lymphoscintigraphy by using technetium-99m nanocolloid as radioactive tracer. During surgery, the sentinel node was identified with triple technique consisting of lymphoscintigraphy, blue dye (Bleu Patente V; Guerbet, Aulnay-sous-Bois, France) and detection of radioactivity by gamma probe.

If an (completion) ALND was performed, the following anatomical landmarks were marked: caudal-medial edge, caudal-lateral edge and cranial-lateral edges of the specimen. A clip was placed at the branching of the lateral thoracic vein from the axillary vein. Finally, the medial apex of axilla was marked.

Histopathological evaluation

For histological evaluation of sentinel nodes, nodes were sliced with a maximum slice thickness of 3 mm and embedded in paraffin. Each part was step sectioned at 500-micrometer intervals at three levels and stained with haematoxylin and eosin (H&E). Cytokeratin immunohistochemical staining, using a mouse anti-human MNF116 antibody (Dako, Glostrup, Denmark), was performed if conventional H&E slices were negative.

For histological evaluation in case of an (completion) ALND, the ALND specimen was received immediately from the operating room. Before formalin fixation, the specimen was fixed in a box representing the normal anatomical situation in the patient. All lymph nodes were embedded in paraffin after 24 hours of fixation. Lymph nodes bigger than 5 mm were sliced with a maximum slice thickness of 3 mm, and stained with H&E.^{14,15}

Each node was recorded as either benign (pNo) or as isolated tumour cells (ITC) (pNo(i+)) (≤ 0.2 mm), micrometastasis (pN1mi) ($0.2 \leq 2.0$ mm) or macrometastasis (pN1-3) (> 2.0 mm). Isolated tumour cells (ITC) and micrometastases were considered negative.

Node-by-node evaluation

A preoperative SPECT/CT scan was used for matching sentinel nodes with axillary MRI, if available. To achieve a reliable node-by-node match between the ALND specimen and MRI, every node harvested by the pathologist was drawn on an anatomical map. This matching procedure was described in detail in earlier publications.^{11,15} The matching of SPECT/CT images and ALND specimens with axillary MR images was performed by two researchers (T.v.N. and R.J.S.) in consensus; both were blinded to pathology findings and T2W-MRI and GDF-MRI results.

Statistical analyses

Statistical analyses were performed using SPSS (version 22.0; IBM, Armonk, New York, USA) and R Project (version 3.3.1; R Foundation for Statistical Computing, Vienna, Austria).

In patients treated with SLNB, lymph nodes on axillary MRI identified as sentinel nodes were taken into account for patient-by-patient analysis only. In patients treated with ALND, all lymph nodes depicted on axillary MRI were included into patient-by-patient analysis.

Diagnostic performance parameters, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under the receiver operating characteristic (ROC) curve (AUC), were analyzed for T2W-MRI and T2W- + GDF-MRI. The cut-off on the confidence level scale was defined between 2 (possibly benign) and 3 (probably malignant), with lymph nodes scored 2 or lower categorized as benign and those scored 3 or higher as malignant.

Since lymph nodes are clustered per patient when reading axillary MRI, lymph node observations within one patient cannot be regarded as independent observations. To adjust for this within-patient correlation, bootstrapping was performed (n=1000 repetitions) for calculation of the 95% confidence intervals for the diagnostic performance parameters.^{15,16}

Differences in diagnostic performance parameters between T2W-MRI and T2W- + GDF-MRI of included patients were analyzed. For patient-by-patient validation a nonparametric test for comparing correlated receiver operating characteristic curves was used.¹⁷

Sample size was based on a previous pilot study investigating GDF-MRI in breast cancer patients.¹¹ With an expected sensitivity of 87% (width of the 95% confidence interval of 9%), a total of 215 lymph nodes with positive test results was required to validate the use of gadofosveset. Based on information of patients treated in our hospital, an average of 1.5 positive lymph nodes was detected in breast cancer patients, resulting in 145 patients. With an expected 10% drop-out, a total of 160 patients were required.

To investigate the presence of a learning curve for reading GDF-MRI, all diagnostic performance parameters of the first one-third nodes was compared to the next and last one-third, on a node-by-node basis.

P-values <0.05 were considered statistical significant.

Results

Patients

During the study period, 97 patients were included and underwent T2W- and GDF-MRI. The study was closed early, because the license of manufacturing gadofosveset in the Netherlands was withdrawn. In all patients who underwent GDF-MRI, no serious adverse events were observed. Seven patients were excluded; one because of withdrawal after signing informed consent, two because of claustrophobia during axillary MRI, one since no axillary surgery was performed, one because histopathology showed chronic lymphocytic leukaemia, one because of silicon adenitis in the lymph nodes, and one because of technical interruption concerning the storage of the MR images. Consequently, results of 90 patients were included in final analysis. Patient and tumour characteristics are summarized in Table 5.1. Seventy-eight patients underwent SLNB or ALND first; the remaining twelve were treated with neoadjuvant systemic therapy. Median time interval between axillary MRI and surgery was seven days (range 0-30 days).

Table 5.1 Patient demographics and tumour characteristics.

Characteristic	Value
Number of Patients	90
Age, years	
Mean (SD)	59 (11)
Range	22-80
Clinical tumour size in situ carcinoma, mm (n=6)	
Mean (SD)	37 (25)
Range	17-85
Clinical tumour size invasive carcinoma, mm (n=84)	
Mean (SD)	21 (11)
Range	6-64
cT-stadium, n (%)	
Tis	6 (7)
T1	48 (53)
T2	33 (37)
T3	3 (3)
pN-stadium, n (%)	
pNo	57 (64)
pNo(i+)	5 (6)
pN1mi	5 (6)
pN1	9 (10)
pN2	4 (4)
pN3	1 (1)
ypNo	4 (4)
ypN1mi	1 (1)
ypN1	1 (1)
ypN2	1 (1)
ypN3	2 (2)

Table 5.1 (continued)

Characteristic	Value
Pathologic tumour type, n (%)	
Ductal carcinoma in situ	6 (7)
Ductal	65 (72)
Lobular	13 (14)
Other	6 (7)
Uni-/multifocal tumours n (%)	
Unifocal	72 (80)
Multifocal and/or multicentric	18 (20)
Hormone receptor and HER2 status, n (%)	
Triple negative	11 (12)
ER/PR + HER2 -	66 (73)
ER/PR - HER2 +	1 (1)
ER/PR + HER2 +	6 (7)
Not determined (in case of in situ carcinoma)	6 (7)

N, number of cases; SD, standard deviation; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

Histopathology

A total of 362 lymph nodes were removed during SLNB (147 nodes) and ALND (215 nodes), of which 263 could be matched to lymph nodes identified on T2W-MRI. Histopathological examination resulted in 75 macrometastases and 188 negative lymph nodes (including 6 ITCs and 16 micrometastases). On patient level, at least one axillary macrometastasis was detected in 20 out of 90 patients. Findings on axillary MRI compared to histopathology are presented in Table 5.2.

Table 5.2 Lymph node scoring on axillary MRI and findings by histopathology.

			Histopathology			
			Benign	Isolated tumour cells	Micrometastasis	Macrometastasis
R1	T2W-MRI	-	149	6	14	36
		+	17	0	2	39
	T2W- + GDF-MRI	-	146	6	12	31
		+	20	0	4	44
R2	T2W-MRI	-	157	6	16	43
		+	9	0	0	32
	T2W- + GDF-MRI	-	152	6	14	38
		+	14	0	2	37

R1, radiologist 1; R2, radiologist 2; T2W-MRI = T2-weighted MRI; GDF-MRI, gadofosveset-enhanced T1-weighted MRI. Absolute numbers are presented.

Diagnostic performance: patient-by-patient analysis

For reader 1 AUC-values of T2W-MRI and T2W-MRI + GDF-MRI were 0.75 and 0.77 ($P=0.731$), for reader 2 0.79 and 0.72 ($P=0.156$). Corresponding diagnostic parameters and 95% confidence intervals (CIs) are presented in Table 5.3.

Diagnostic performance: node-by-node analysis

For reader 1, T2W-MRI showed an AUC of 0.76 and T2W-MRI + GDF-MRI an AUC of 0.82 ($P=0.018$). For reader 2 AUC of both T2W-MRI and T2W-MRI + GDF-MRI was 0.77 ($P=0.997$), (Table 5.3).

Table 5.3 Diagnostic performance for prediction of axillary nodal status.

	R1		R2	
	T2W-MRI	T2W- + GDF-MRI	T2W-MRI	T2W- + GDF-MRI
Node-by-node (prevalence of metastasis: 75/263)				
Sensitivity	52 (39/75)	59 (44/75)	43 (32/75)	49 (37/75)
95% CI	40-63	48-69	31-55	39-60
Specificity	90 (169/188)	87 (164/188)	95 (179/188)	92 (172/188)
95% CI	86-94	82-91	92-98	88-95
PPV	67 (39/58)	65 (44/68)	78 (32/41)	70 (37/53)
95% CI	57-78	56-74	66-89	59-80
NPV	82 (169/205)	84 (164/195)	81 (179/222)	82 (172/210)
95% CI	79-86	81-88	77-84	79-85
AUC	0.76	0.82	0.77	0.77
95% CI	0.69-0.83	0.76-0.87	0.70-0.84	0.71-0.83
P Value [†]	0.018		0.998	
Patient-by-patient (prevalence of metastasis: 20/90)				
Sensitivity	40 (8/20)	40 (8/20)	30 (6/20)	35 (7/20)
95% CI	20-64	20-64	13-54	16-59
Specificity	90 (63/70)	84 (59/70)	94 (66/70)	91 (64/70)
95% CI	80-96	73-92	85-98	82-96
PPV	53 (8/15)	42 (8/19)	60 (6/10)	54 (7/13)
95% CI	27-78	21-66	27-86	26-80
NPV	84 (63/75)	83 (59/71)	83 (66/80)	83 (64/77)
95% CI	73-91	72-91	72-90	72-90
AUC	0.75	0.77	0.79	0.72
95% CI	0.62-0.88	0.66-0.88	0.67-0.90	0.58-0.86
P Value [‡]	0.731		0.156	

R1, radiologist 1; R2, radiologist 2; T2W-MRI = T2-weighted MRI; GDF-MRI, gadofosveset-enhanced T1-weighted MRI; 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; numbers are percentages, absolute numbers are given in parentheses.

[†] *P Value* for difference in AUC between T2W- and GDF-MRI, based on bootstrap test ($n=1000$). [‡] *P Value* for difference in AUC between T2W- and GDF-MRI, based on DeLong's test.

Learning curve analysis

For reader 1, AUC of T2W-MRI + GDF-MRI of the first 88 nodes was 0.71, while the AUC of the following 88 nodes was 0.80 and the AUC of the last 87 nodes was 0.95. The AUC-values of reader 2 were 0.69, 0.80 and 0.79. Corresponding ROC curves of the AUC are presented in Figure 5.3, and all corresponding diagnostic parameters are presented in Table 5.4.

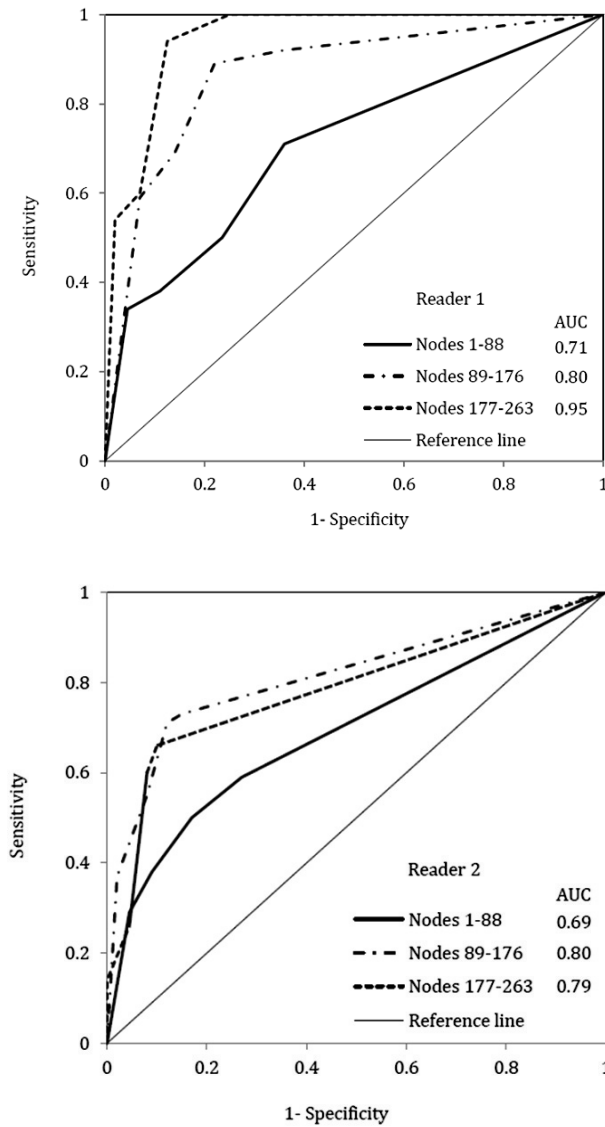


Figure 5.3 Receiver operating characteristics curves and areas under the curve (AUC) based on node-by-node analysis for detection of metastatic lymph nodes in the first 88 (bold line), next 88 (dash dotted line) and last 87 (dotted line) nodes, using the combination of T2-weighted (T2W) and gadofosveset-enhanced T1-weighted (GDF) MRI for reader 1 (a) and 2 (b).

Table 5.4 Diagnostic performance for prediction of axillary nodal status node-by-node

	R1 T2W- + GDF-MRI			R2 T2W + GDF-MRI		
	Nodes 1-88	Nodes 89-176	Nodes 177-263	Nodes 1-88	Nodes 89-176	Nodes 177-263
Sensitivity	38 (9/24)	72 (26/36)	60 (9/15)	38 (9/24)	67 (24/36)	27 (4/15)
95% CI	21-58	58-86	33-87	21-54	50-81	7-47
Specificity	89 (57/64)	77 (40/52)	93 (67/72)	91 (58/64)	87 (45/52)	96 (69/72)
95% CI	81-95	65-88	86-99	83-97	77-94	90-100
PPV	56 (9/16)	68 (26/38)	64 (9/14)	60 (9/15)	77 (24/31)	57 (4/7)
95% CI	35-79	58-81	44-88	37-85	66-90	17-100
NPV	79 (57/72)	80 (40/50)	92 (67/73)	79 (58/73)	79 (45/57)	86 (69/80)
95% CI	74-85	72-89	87-97	74-85	71-87	83-90
AUC	0.71	0.80	0.95	0.69	0.80	0.79
95% CI	0.58-0.82	0.70-0.88	0.90-0.98	0.56-0.80	0.71-0.89	0.66-0.92

R1, radiologist 1; T2W-MRI = T2-weighted MRI; GDF-MRI, gadofosveset-enhanced T1-weighted MRI; R2, radiologist 2; 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; numbers are percentages, absolute numbers are given in parentheses.

Discussion

The aim of this prospective study was to evaluate the diagnostic performance of gadofosveset-enhanced MRI for nodal (re)staging in breast cancer. Both readers showed comparable diagnostic performance of T2W-MRI (per node AUC of 0.76 and 0.77). The addition of GDF-MRI improved the performance for reader 1 to 0.82 ($P=0.018$), while reader 2 had no benefit of GDF-MRI compared to T2W-MRI.

To understand whether a learning curve existed for GDF-MRI, both readers had feedback on their errors after each axillary MRI. The reader who had the longest experience in nodal staging with GDF-MRI (reader 1) improved in performance after addition of GDF-MRI to T2W-MRI, from an AUC of 0.71 for the first 88 nodes to 0.95 for the last 87 nodes. The breast imaging specialized radiologist (reader 2) did not seem to improve in performance. This suggests that an individual learning curve exists.

In this study, two radiologists with different levels of experience in breast cancer imaging and nodal staging participated. The diagnostic performances of T2W-MRI for both expert (reader 2) and non-expert (reader 1) in breast imaging are in line with previous reports on nodal staging in breast cancer with standard T2W-MR sequences.¹⁸ This suggests that radiologists – whether or not specialized in breast cancer imaging, do well with nodal staging at standard T2W-MRI. Yet, this remains insufficiently accurate to replace SLNB, with reported NPVs of 84% or lower in our study.

Of interest is the difference in performance using the combined T2W-MRI + GDF-MRI of the two radiologists after the first 176 nodes. In the last 87 nodes of our cohort, reader 1 showed a substantial improvement after addition of GDF-MRI to T2W-MRI, from an AUC of 0.71 to 0.95, equal to reported figures of rectal cancer MR nodal staging using GDF-MRI.^{19,20} Reader 2 however, did not seem to improve. A possible explanation might be that reader 1 was inexperienced in breast imaging interpretation, but had extensive experience in rectal cancer nodal staging with MRI, including 8 years of interpreting gadofosveset-enhanced MRIs. Therefore, reader 1 was more aware of interpretation difficulties with GDF-MRI and of own pitfalls, because of previous experience and continuous feedback in rectal cancer.¹⁹ Although reader 2 is an expert breast radiologist, he was not familiar with GDF-MRI, which might explain differences in performances.

The diagnostic performance of T2W-MRI and T2W-MRI + GDF-MRI was calculated based on histopathology of surgically removed lymph nodes. Lymph nodes containing ITCs and micrometastases were recorded as benign findings. However, the clinical relevance of these small metastases is limited.²¹⁻²⁵ Therefore, ignoring micrometastases and ITCs is justified.

Axillary ultrasound combined with ultrasound-guided biopsy if indicated, shows high specificity (98.3%) and high PPV (97.1%).²⁶ The PPV of GDF-MRI cannot compete with that of ultrasound-guided biopsy, as it is not higher than 78%. Therefore, our study showed that GDF-MRI cannot replace ultrasound-guided nodal biopsy to confirm positive lymph nodes.

Of special interest is the NPV of 92% of GDF-MRI, suggesting that GDF-MRI can identify (y)pNo patients. This NPV of GDF-MRI outperforms the NPV of axillary ultrasound.²⁶⁻²⁸ Furthermore, an NPV of 92% approximates the NPV of 96% of the more invasive SLNB.²⁹ It also outperforms the diagnostic performance of SLNB post-neoadjuvant systemic therapy or the MARI-procedure in clinically node positive breast cancer patients.³⁰⁻³² Unfortunately, due to the relatively low sensitivity of GDF-MRI (27-60%) it will remain challenging for implementation of GDF-MRI into clinical practice.

This study is limited by the relatively small number of patients. According to sample size calculation, a total of 215 positive lymph nodes with an expected sensitivity of 87% was required. However, in this study we only included a total of 75 positive lymph nodes, since this study was closed early. Furthermore, the difference in sensitivity between a previous pilot study and this study for reading GDF-MRI (86% versus 59%) can be explained by different inclusion criterion: clinically palpable nodes or suspicious nodes with axillary ultrasound in the pilot study versus breast cancer patients without any information for the readers

concerning nodal status in this study. Next, the results of this study are highly dependent on reader's performance. Consequently, reproducibility of these results by less experienced readers could be challenging. Finally, predictive values (PPV and NPV) for investigating T2W- and GDF-MRI depend on prevalence of axillary macrometastases in this study. Therefore, results should be interpreted with the prevalences of this cohort in mind, which consisted of 28.5% (node-by-node) and 22.2% (patient-by-patient).

The current study confirmed previous study results that GDF-MRI, in addition to T2W-MRI, has potential as a noninvasive method for nodal (re)staging in breast cancer.

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Chapter 6

**Added value of dedicated axillary hybrid
18F-FDG PET/MRI to improve axillary nodal
staging in clinically node positive breast
cancer patients – a feasibility study**

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Abstract

Objective

To investigate the feasibility and potential added value of dedicated axillary ^{18}F -FDG hybrid PET/MRI, compared to standard imaging modalities (i.e. ultrasound (US), MRI and PET/CT) for axillary nodal staging in clinically node-positive breast cancer.

Methods

Twelve patients with clinically node-positive breast cancer underwent axillary US and dedicated axillary hybrid ^{18}F -FDG PET/MRI. Nine out of these twelve patients also underwent whole-body PET/CT. Maximum standardized uptake values (SUVmax) were measured for the primary breast tumour and the most FDG-avid axillary lymph node. A positive axillary lymph node on dedicated axillary hybrid PET/MRI was defined as a moderate to very intense FDG-avid lymph node. The diagnostic performance of dedicated axillary hybrid PET/MRI was calculated by comparing quantitative and its qualitative measurements to results of axillary US, MRI and PET/CT. The number of suspicious axillary lymph nodes was subdivided as follows: No (0 nodes), N₁ (1-3 nodes), N₂ (4-9 nodes) and N₃ (≥ 10 nodes).

Results

According to dedicated axillary hybrid PET/MRI findings, seven patients were diagnosed with N₁, four N₂ and one N₃. With regard to mean SUVmax, there was no significant difference of primary tumour ($9.0 (\pm 5.0)$ versus $8.6 (\pm 5.7)$, $P=0.678$) or the most FDG-avid axillary lymph node ($7.8 (\pm 5.3)$ versus $7.7 (\pm 4.3)$, $P=0.767$) between dedicated axillary PET/MRI and PET/CT. Compared to standard imaging modalities, dedicated axillary hybrid PET/MRI resulted in changes in nodal status as follows: 40% compared to US, 75% compared to T₂ weighted MRI, 40% compared to contrast-enhanced MRI and 22% compared to PET/CT.

Conclusion

Adding dedicated axillary ^{18}F -FDG hybrid PET/MRI to diagnostic work-up may improve diagnostic work-up of axillary nodal staging in clinically node-positive breast cancer patients.

Introduction

Breast cancer is one of the most prevalent types of invasive cancer among women.¹ The involvement of axillary lymph nodes is an important prognostic factor, as 5-year overall survival is reduced from 98% to 85% in case of lymph node metastases.² Therefore, European guidelines recommend preoperative axillary imaging to differentiate between patients with and without clinically node-positive breast cancer.³ Accurate lymph node staging is important, as it affects the type of axillary surgery and indications for systemic therapy and radiotherapy fields.⁴

In addition to differentiating between node-negative and node-positive disease, an equally important consideration is the number of suspicious nodes. Current treatment plans are based on the number of suspicious lymph nodes (i.e. one to three versus four or more) according to imaging findings and histopathological confirmation prior to start of therapy.³ Preoperative axillary imaging currently involves the use of axillary ultrasound (US) with tissue sampling in case of suspicious nodal findings. Schipper et al. demonstrated that more than 50% of patients with one to three suspicious lymph nodes on US had four or more lymph node metastases at surgery.⁵ This percentage decreased to 11-15% when standard breast MRI was used for axillary nodal staging.⁶ In addition, the use of a dedicated axillary MR protocol in place of a standard breast MR protocol further improves diagnostic performance of MRI for nodal staging.⁷ Research suggests that PET/CT has the highest accuracy for preoperative nodal staging: Koolen et al. demonstrated that 98% of all patients with suspicious axillary lymph nodes on PET/CT would eventually have axillary lymph node metastases after surgery.⁸

Hybrid PET/MRI allows for the simultaneous use of the promising axillary nodal staging techniques PET and MRI.⁹ Therefore, the aim of this study was to investigate the feasibility and potential added value of dedicated axillary 18F-FDG hybrid PET/MRI compared to standard imaging modalities (i.e. axillary US, MRI and PET/CT) for axillary nodal staging in patients with clinically node-positive breast cancer.

Materials and methods

Setting and patients

After approval of the study by the local medical ethical committee, a total of twelve patients provided signed informed consent to undergo dedicated axillary hybrid PET/MRI. Inclusion criteria were patients with histopathologically

confirmed clinically node-positive breast cancer. Exclusion criteria were contraindications for either MRI, including contrast agent gadobutrol, or PET, including radiotracer ^{18}F -fluorodeoxyglucose (^{18}F -FDG). Prior to inclusion, patients underwent axillary US. In addition, all patients were planned for MR imaging and, depending on number of suspicious lymph nodes (i.e. one to three versus four or more), for PET/CT as well.¹⁰

Axillary ultrasound

Axillary US exams were performed by the radiologist on call, using an ultrasound system with a linear 2 to 17 MHz array transducer (iU22-xMATRIX, Philips Healthcare, Best, the Netherlands). Criteria for suspicious axillary lymph nodes included diffuse cortical thickening, focal cortical mass and/or thickening and loss of fatty hilum.¹¹ During axillary US, tissue sampling of suspicious axillary lymph nodes was performed. In cases of multiple suspicious nodes, only the most suspicious node was sampled, and the number of suspicious axillary lymph nodes was reported.

PET/CT

In the Netherlands, PET/CT exams can be considered in case of stage III/IV breast cancer.^{10,12} After patients had fasted for a fasting period of at least four hours and had a blood glucose level <10 mmol/L, an intravenous injection of ^{18}F -FDG was administered with a dose of 2 MBq/kg bodyweight, followed by a resting period of 60 \pm 10 minutes. Patients were placed in supine position with elevated arms in the PET/CT scanner (Gemini TF, Philips Healthcare, Best, the Netherlands). A low-dose CT scan (120 kV, 30 mAs, slice thickness 4 mm) from head to thigh was performed, followed by the PET acquisition (3 minutes per bed position). CT images were reconstructed using filtered back projection. PET images were reconstructed using the BLOB-OS-TF time-of-flight algorithm provided by the manufacturer, with a voxel size of 4 x 4 x 4 mm³.

Hybrid PET/MRI

Directly after whole-body PET/CT, state-of-the-art dedicated axillary hybrid PET/MRI images were acquired on a 3.0T Biograph mMR integrated PET/MRI system (Siemens Healthineers, Erlangen, Germany). Dedicated axillary MRI was performed using a six-channel anterior body coil (Siemens Healthineers), which was placed on the ipsilateral shoulder. Patients were placed in prone position with both arms elevated.

In three cases no whole-body PET/CT was performed. These patients underwent dedicated axillary hybrid PET/MRI after administration of ^{18}F -FDG

with a dose of 2 MBq/kg bodyweight and a resting period of 60 +/- 10 minutes, with identical fasting time and a blood glucose level < 10 mmol/L as with PET/CT.

The dedicated axillary hybrid PET/MRI protocol consisted of an unenhanced T2-weighted (T2W) sequence, contrast-enhanced T1-weighted sequence (T1W) and a fusion sequence of PET-images with the unenhanced T2W sequence. All sequences were performed in coronal view.

More specifically, a 2D standard T2 weighted (T2W) sequence without fat suppression was performed first using the following scan parameters: repetition time (TR) 2000 ms; echo time (TE) 118 ms; variable degree flip angle; turbo spin echo (TSE) factor 41; averages 1.4; voxel size 1.1 x 1.1 x 1.1 mm; field of view (FOV) 220 x 220 mm²; matrix 192 x 192, resulting in an in-plane resolution of 1.15 x 1.15 mm². Next, gadobutrol (Gadovist®, Bayer HealthCare, Berlin, Germany) contrast with an amount of 15 cc was administered intravenously. After 6 minutes a 3D T1 weighted (T1W) sequence was acquired using the following parameters: TR 7.27 ms; TE 2.46 ms; 15 degree flip angle; averages 2; voxel size 1.1 x 1.1 x 1.1 mm; FOV 220 x 220 mm²; matrix 192 x 192; resulting in an in-plane resolution of 1.15 x 1.15 mm². Total acquisition time of the MRI exam was 20 minutes and 21 seconds.

Three-dimensional iterative reconstruction was performed for all PET images of the hybrid PET/MRI system (number of iterations 3; subsets 21; voxel size 4.1 x 2.6 x 3.9 mm³; image matrix 172 x 172; zoom 1.0) and automatic attenuation correction through implementation of a four-compartment model attenuation map (Dixon based μ -map). Images were acquired in a single bed position within 15 minutes after start of the MRI acquisition on the hybrid PET/MRI system.

Image evaluation

All axillary lymph nodes on dedicated axillary MR images, hybrid PET/MRI and PET/CT images were evaluated by a breast radiologist (M.B.I.L.) and nuclear medicine physician (S.V.), respectively. The breast radiologist has 7 years of dedicated experience in breast imaging, whereas the nuclear medicine physician has 9 years of experience in nuclear imaging.

First, the radiologist scored each axillary lymph node on dedicated axillary T2W images, according to the criteria previously defined by Baltzer et al., using a confidence scale from 0 (no lymph nodes) to 4 (definitely malignant).¹³ Lymph nodes with a score of 0-2 were considered benign, and those with a score of 3-4 malignant. Suspicious characteristics included irregular margins, inhomogeneous cortex, perifocal edema, absent fatty hilum, asymmetry, and absence of chemical shift artifacts.¹³⁻¹⁵ Next, contrast-enhanced dedicated axillary T1W images were shown, which allowed the radiologist to adjust his score on each lymph node, if

deemed necessary. Suspicious characteristics included absence of contrast hyperintensity of the lymph node and absence of an intact nodal border.¹⁵

Second, the breast radiologist and nuclear medicine physician evaluated in consensus each axillary lymph node on dedicated axillary hybrid PET/MRI using a four-point system as previously described by Aukema et al, for the assessment of lymph nodes with ¹⁸F-FDG PET/CT: 0 = similar to other surrounding lymph nodes, 1 = slightly more intense than other lymph nodes, 2 = moderate intense and 3 = very intense.¹⁶ Lymph nodes with a score of 0-1 FDG uptake were considered benign, while a score of 2-3 FDG uptake was considered malignant. PET information was considered to be decisive regarding the number of suspicious axillary lymph nodes in the evaluation of dedicated axillary hybrid PET/MRI. The nuclear medicine physician measured further maximum standardized uptake values (SUVmax) of the primary breast tumour and the most FDG-avid axillary lymph node on each dedicated axillary hybrid PET/MRI exam. Finally, the nuclear medicine physician evaluated each axillary lymph node on ¹⁸F-FDG PET/CT, using the same four-point system,¹⁶ and measured SUVmax of the primary breast tumour and the most FDG-avid axillary lymph node on each PET/CT exam. Both PET/CT and PET/MRI exams were shown anonymously and in randomized order to the nuclear physician for evaluation of the number of suspicious axillary lymph nodes.

Pathology

In this study, clinically node-positive disease was based on US-guided cytological and/or histological confirmation of axillary lymph node biopsy. In cases where US was performed in the referring hospital, pathology of the axillary lymph node biopsy was revised and metastases were verified by the pathologist in our hospital (L.K.).

Statistical analyses

Statistical analyses were performed using SPSS software (version 22, IBM, Armonk, New York, USA). Axillary nodal status was based on number of suspicious axillary lymph nodes: none (No), 1-3 (N₁), 4-9 (N₂) or 10 or more (N₃), according to axillary US (including results of tissue sampling), dedicated axillary (PET)MRI and/or PET/CT, respectively. When the result of dedicated axillary hybrid PET/MRI led to either up- or downstaging of clinical nodal status compared to standard imaging modalities (US, MRI or PET/CT), it was considered a change in clinical nodal status according to dedicated axillary hybrid PET/MRI findings.

In patients who underwent both PET/CT and hybrid PET/MRI, Wilcoxon signed rank test was used to compare the mean SUVmax of primary tumour

(\pm standard deviation (SD)) and the most FDG-avid axillary lymph node (\pm SD) of PET/CT with hybrid PET/MRI. *P*-values (two-sided) <0.05 were considered statistically significant.

Results

Patients

A total of twelve consecutive patients with clinically node-positive breast cancer over a period from April 2016 until March 2017 were included in this feasibility study. The mean age was 49 years (range, 28 – 70 years). In all patients, clinically positive nodal status was histopathologically confirmed with US-guided biopsy. Eleven out of twelve patients had invasive carcinoma of no special type, one patient had mixed lobular carcinoma with carcinoma of no special type. In addition, nine out of twelve patients underwent PET/CT. A more detailed overview of the general characteristics is shown in Table 6.1.

Table 6.1 General patient characteristics.

Mean age (years) (range)	49 (28 – 70)
Mean clinical tumour size (mm) (range)	46 (13 – 150)
cT-stage (%)	
cT1	2 (17%)
cT2	4 (33%)
cT3	3 (25%)
cT4	3 (25%)
Multifocal tumour (%)	
No	8 (67%)
Yes	4 (33%)
Tumour type (%)	
Invasive carcinoma NST	11 (92%)
Mixed ductal and lobular	1 (8%)
Tumour grade (%)	
2	6 (50%)
3	6 (50%)
Hormonal and receptor status	
ER/PR positive, Her2 negative	4 (33%)
ER/PR negative, Her2 positive	1 (8%)
Triple negative	2 (17%)
ER/PR positive, Her2 positive	5 (42%)

Abbreviations: *cT-stage* clinical tumour stage, *NST* no special type, *ER* estrogen, *PR* progesterone, *HER2* Human Epidermal growth factor Receptor 2.

Nodal staging

When compared to standard imaging modalities, dedicated axillary hybrid PET/MRI resulted in changes to clinical nodal status as follows: 40% according to US findings (4 out of 10 patients), 75% according to T2W-MRI findings (9 out of 12 patients), 40% according to CE-T1W MRI findings (4 out of 10 patients) and 22% according to PET/CT findings (2 out of 9 patients), respectively. Differences between PET/CT and PET/MRI findings were due to better delineation of the lymph nodes on the MRI component. A more detailed overview of all cases, including up- or downstaging of nodal status according to dedicated axillary hybrid PET/MRI findings, is provided in Table 6.2.

In two patients axillary US findings regarding the number of suspicious axillary lymph nodes was missing, because US was performed in the referring hospital. However, in addition, neither patient underwent contrast-enhanced T1-weighted MRI, as this was refused by both patients.

Figures 6.1 and 6.2 illustrate examples of differences in number of suspicious axillary lymph nodes between PET/CT (Figure 6.1) and T2W-MRI (Figure 6.2) when compared to dedicated axillary hybrid PET/MRI. Appendix 1 provides a complete overview of all axillary lymph nodes evaluated on dedicated axillary (PET/)MRI and PET/CT images.

Table 6.2 Clinical nodal status (including number of suspicious lymph nodes) according to US, PET/CT, T2W-MRI, CE-T1W-MRI and hybrid PET/MRI.

Patient	US	PET/CT	Dedicated axillary (PET/)MRI		
			T2W-MRI	CE-T1W MRI	Hybrid PET/MRI
1	N2 (4)	N2 (5)	N3 (10) ↓	N3 (10) ↓	N2 (8)
2	N1 (1)	-	No ↑	N1 (1)	N1 (2)
3	N1 (1)	-	N2 (5) ↓	N2 (7) ↓	N1 (1)
4	N1 (3) ↑	N2 (5)	N3 (10) ↓	N2 (9)	N2 (8)
5	N+ (-)	N2 (9)	N1 (3) ↑	-	N2 (7)
6	N2 (6) ↓	N1 (2)	N3 (25) ↓	N3 (11) ↓	N1 (2)
7	N+ (-)	N2 (4) ↓	N2 (4) ↓	-	N1 (3)
8	N1 (1)	N1 (3)	N2 (6) ↓	N2 (7) ↓	N1 (3)
9	N2 (5) ↑	N2 (7) ↑	N3 (10)	N3 (13)	N3 (10)
10	N1 (1)	N1 (1)	N1 (2)	N1 (2)	N1 (1)
11	N1 (2) ↑	-	N1 (3) ↑	N2 (5)	N2 (4)
12	N1 (1)	N1 (1)	N1 (2)	N1 (3)	N1 (1)

Abbreviations: PET Positron Emission Tomography, CT Computed Tomography, MRI Magnetic Resonance Imaging, T2W T2-weighted, CE-T1W contrast-enhanced T1-weighted, No no suspicious lymph nodes, N1 1-3 suspicious lymph nodes, N2 4-9 suspicious lymph nodes, N3 10 or more suspicious lymph nodes, N+ clinically node positive status, - missing, ↓ downstaging according to result of hybrid PET/MRI, ↑ upstaging according to result of hybrid PET/MRI.

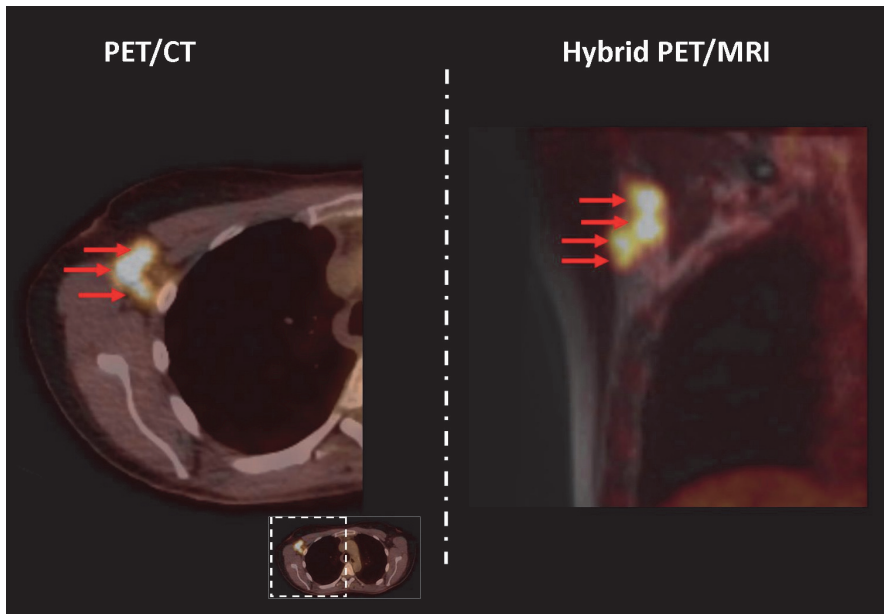


Figure 6.1 Example of a patient with a 35 mm invasive carcinoma of no special type in her right breast. One cross-sectional PET/CT image (left) of the right axilla demonstrates three suspicious lymph nodes (red arrows) out a total of five suspicious lymph nodes on PET/CT. One coronal dedicated axillary hybrid PET/MRI image (right) demonstrates four suspicious lymph nodes (red arrows), with a total of eight suspicious lymph nodes on dedicated axillary hybrid PET/MRI. The most dorsally located suspicious lymph node on (low-dose) PET/CT consists of two suspicious lymph nodes on dedicated axillary hybrid PET/MRI.

SUVmax measurements

The mean SUVmax of the primary tumour and the most FDG-avid axillary lymph node on PET/CT were $8.6 (\pm 5.7)$ and $7.7 (\pm 4.3)$, respectively. Comparable values were achieved on hybrid PET/MRI, with mean SUVmax of primary tumour $9.0 (\pm 5.0)$ ($P=0.678$) and mean SUVmax of the most FDG-avid lymph node $7.8 (\pm 5.3)$ ($P=0.767$). Table 6.3 provides an overview of all SUVmax measurements on PET/CT and hybrid PET/MRI for each patient.

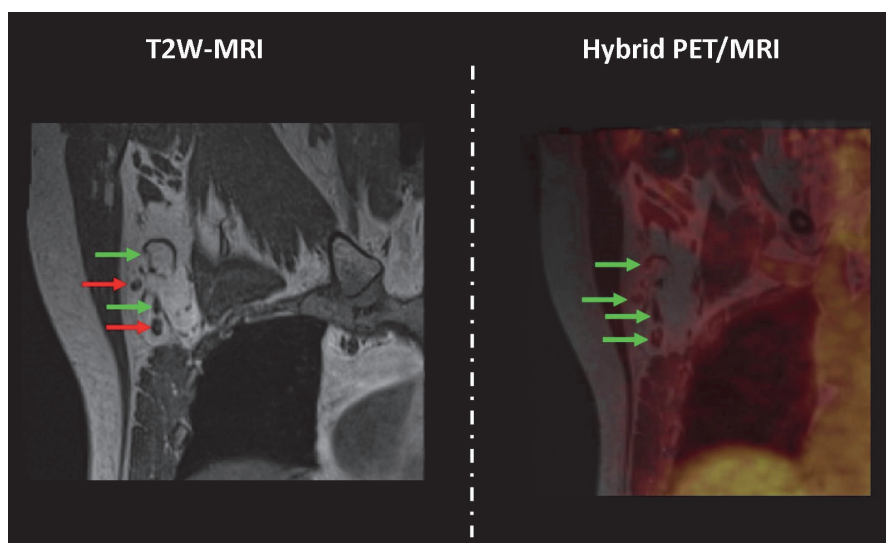


Figure 6.2 Example of a patient with a 31 mm invasive carcinoma of no special type in her right breast. A coronal T2-weighted MR image (left) of the right axilla demonstrates two out of four (red arrows) suspicious nodes. A coronal dedicated axillary hybrid PET/MRI image (right) demonstrates no FDG-uptake in any of the four nodes, which resulted in four negative nodes on dedicated axillary hybrid PET/MRI.

Discussion

The aim of this study was to investigate the feasibility and potential added value of dedicated axillary ^{18}F -FDG hybrid PET/MRI compared to standard imaging modalities (i.e. US, MRI and PET/CT) for axillary nodal staging in patients with clinically node-positive breast cancer. The results of our study show that dedicated axillary ^{18}F -FDG hybrid PET/MRI is clinically feasible, and resulted in a change in nodal status in 40-75% of patients when compared to US or MRI. When compared to PET/CT only, nodal status was changed in two out of nine patients (22%), although SUVmax measurements were comparable between the imaging modalities.

The importance of accurate differentiation in clinically node-positive patients, i.e. one to three versus four or more positive nodes, prior to start of treatment is a topic of debate since adjuvant (radiation) therapy planning is based on pre-treatment axillary imaging findings in the case of neoadjuvant systemic therapy.¹⁷ With regard to patients with limited axillary disease after surgery, the

ACOSOG Z0011 trial demonstrated that there was no prognostic benefit in adjuvant axillary treatment in clinically node-negative patients treated with primary surgery.¹⁸ On the other hand, patients with advanced axillary disease (i.e. four or more positive nodes) do require adjuvant regional treatment.^{17,19} Consequently, pre-treatment axillary imaging is becoming increasingly important as a means of truly identifying patients with four or more versus three or fewer positive nodes. However, long-term outcome for patients treated with additional therapy after the detection of a larger number of positive – for instance, on PET/CT as opposed to ultrasound – remains unclear.

Table 6.3 Per patient overview of SUVmax measurements on PET/CT and hybrid PET/MRI.

Patient	PET/CT <i>SUVmax</i>	Hybrid PET/MRI <i>SUVmax</i>
1		
Primary tumour	9.25	12.00
Axillary lymph node	8.70	11.03
2		
Primary tumour	11.10	11.70
Axillary lymph node	16.00	20.05
3		
Primary tumour	8.80	7.60
Axillary lymph node	7.70	4.60
4		
Primary tumour	5.10	10.30
Axillary lymph node	1.95	1.70
5		
Primary tumour	19.50	17.10
Axillary lymph node	11.90	8.50
6		
Primary tumour	3.40	3.90
Axillary lymph node	6.60	5.70
7		
Primary tumour	1.80	1.50
Axillary lymph node	4.30	5.40
8		
Primary tumour	3.95	4.45
Axillary lymph node	3.70	4.95
9		
Primary tumour	14.05	12.05
Axillary lymph node	7.90	7.95
Mean		
Primary tumour	8.55	8.96
Axillary lymph node	7.65	7.76

Abbreviations: *PET* Positron Emission Tomography, *CT* Computed Tomography, *MRI* Magnetic Resonance Imaging, *SUVmax* maximum standard uptake value.

In contrast to our study, Grueneisen et al. found no differences of PET/MRI findings compared to PET/CT and MRI for axillary nodal staging in breast cancer patients.²⁰ However, in that study, a (standard) breast PET/MRI protocol was used, unlike the state-of-the-art dedicated axillary PET/MRI protocol in our study. In addition, a previous study demonstrated that in approximately 40% of all patients who underwent standard breast MRI, the field of view of the axillary region was not complete.⁶ In contrast to Grueneisen, a study by Melsaether et al. showed improved diagnostic accuracy of PET/MR imaging versus PET/CT for axillary nodal staging in breast cancer patients, with an improvement in sensitivity from 88% to 100%.²¹ Nonetheless, their study differentiated only node-negative from node-positive disease, rather than considering the number of positive nodes. The results of our study can thus be considered to provide added clinical value compared to standard imaging modalities (US, MRI and PET/CT), further improving a patient-tailored approach for axillary nodal staging in clinically node-positive breast cancer.

The accuracy of MRI for axillary nodal staging in our study may differ from that in standard clinical practice. We used dedicated (coronal) axillary MRI instead of standard (cross-sectional) breast MRI that is most commonly used in clinical practice, which might have resulted in higher accuracy than with standard breast MRI. A previous systematic review by Kuijs et al. similarly demonstrated superior results for axillary nodal staging when a dedicated axillary MRI protocol was used.⁷ However, our study revealed that dedicated axillary hybrid PET/MRI is of added value even compared to dedicated axillary T2W- and contrast-enhanced MRI, as it still resulted in changes to clinical nodal status in respectively 75% and 40% of cases, respectively. In addition, differences in the performance of PET/CT can occur when PET/CT is performed with hanging breast acquisition rather than standard PET/CT. Teixeira et al. demonstrated that PET/CT with hanging breast acquisition improved detection of regional lymph node metastases over standard PET/CT.²²

SUVmax measurements of primary tumour (8.6 versus 9.0) and the most FDG-avid axillary lymph node (7.7 versus 7.8) in our study were comparable between PET/CT and hybrid PET/MRI, despite different reconstruction protocols between both imaging modalities. Pujara et al. also found a strong correlation in SUVmax between PET/CT and PET/MRI in breast cancer patients, and concluded that SUVs from PET/MRI can be used for quantification of ¹⁸F-FDG activity.²³ However, SUVmax comparisons between FDG-avid axillary lymph nodes on PET/CT and PET/MRI were not reported in their study. The results of our study can thus support the earlier findings of Pujara et al. for using SUVmax of FDG-avid axillary nodes on PET/MRI for ¹⁸F-FDG activity quantification.

In the future, whole-body PET/MRI, including an integrated dedicated axillary PET/MRI protocol, might replace whole body PET/CT for locoregional and distant staging in breast cancer, since both locoregional and distant staging seem to be at least as accurate as PET/CT.^{20,24-26} A secondary benefit would be the reduction in radiation exposure to patients of up to 64% compared to PET/CT.²¹ The cost-effectiveness of PET/MRI must also be further investigated.

Limitations of the current study include the missing data concerning correlation of dedicated axillary hybrid PET/MR images and final histopathology of axillary lymph nodes; however, nodal metastases were histopathologically confirmed in all patients. Moreover, FDG-avid axillary lymph nodes can be considered malignant, since previous studies have reported high specificity of 95 – 100% for the detection of axillary lymph node metastases in breast cancer.^{16,27,28} However, because of the lack of initial histopathology in these cases, it is possible that some benign lymph nodes according to FDG-activity on PET/MRI still could have been malignant.

Second, there was a timing difference in the performance of PET/MRI in our cohort. Three out of twelve patients underwent only PET/MRI, instead of PET/CT following PET/MRI. This might have led to differences in SUVmax measurements on PET/MRI between the two subgroups.¹² These three patients were thus excluded from subgroup analysis regarding SUVmax measurements on PET/MRI and PET/CT.

Third, patients underwent unenhanced low-dose whole body PET/CT rather than diagnostic contrast-enhanced PET/CT. The results of PET/CT should be interpreted with this important limitation in mind.

Finally, hybrid PET/MRI is currently available in only a few hospitals worldwide. Therefore, other techniques should also be further explored to approximate the diagnostic performance of PET/MRI, for instance by combining hanging breast PET images with MRI images.²⁹

In conclusion, this feasibility study demonstrated that, compared to standard imaging modalities, dedicated axillary 18F-FDG hybrid PET/MRI appears to improve diagnostic performance for axillary nodal staging in clinically node-positive breast cancer patients. Further studies are needed to investigate the accuracy of this hybrid modality and to compare its performance with that of other dedicated imaging tools, such as hanging breast PET/CT.

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Appendix 6.1

Complete overview of all axillary lymph nodes evaluated on (PET)/MRI and PET/CT exams.

	T2W-MRI		CE-T1W-MRI		Hybrid PET/MRI		PET/CT	
	Benign (score 0-2)	Suspicious (score 3-4)	Benign (score 0-1)	Suspicious (score 2-3)	Benign (score 0-1)	Suspicious (score 2-3)	Benign (score 0-1)	Suspicious (score 2-3)
Patient								
1	2	10	2	10	4	8	1	5
2	11	0	10	1	9	2	-	-
3	15	5	13	7	19	1	-	-
4	2	10	3	9	4	8	0	5
5	4	3	-	-	0	7	2	9
6	3	25	17	11	26	2	5	2
7	6	4	-	-	7	3	0	4
8	9	6	8	7	12	3	0	3
9	4	10	1	13	4	10	0	7
10	9	2	9	2	10	1	0	1
11*	5	3	5	5	6	4	-	-
12	4	2	3	3	5	1	0	1

* This concerned the patient with mixed lobular carcinoma with carcinoma of no special type.
Abbreviations: *T2W* T2-weighted, *MRI* magnetic resonance imaging, *CE-T1W* contrast-enhanced T1-weighted, *PET* Positron Emission Tomography, *CT* Computed Tomography.

Part III

Classification of lymph node staging

Chapter 7

TNM classification and the need for revision of
pN₃a breast cancer

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Abstract

Background

According to the seventh edition of tumour-node-metastasis (TNM) classification, pN3a status in breast cancer patients consists of presence of an infraclavicular lymph node metastasis (LNM) and/or presence of ≥ 10 axillary LNMs. The aim of this study was to determine whether prognosis of pN3a based on at least an infraclavicular LNM differs from ≥ 10 axillary LNMs.

Methods

Data were obtained from the Netherlands Cancer Registry. All patients were diagnosed between 2005 and 2008 with primary invasive epithelial breast cancer and pN2a or pN3a status as pathologic result. Patients with pN3a were subdivided in pN3a based on at least an infraclavicular LNM or ≥ 10 axillary LNMs. Disease-free survival (DFS) included any local, regional or contralateral recurrence, distant metastasis or death within 5 years. Kaplan-Meier curves provided information on 5-year DFS and 8-year overall survival (OS). In addition, Cox proportional hazards model was used to measure the effect of relevant clinicopathological variables on DFS and OS.

Results

A total of 3400 patients with pN2a and 1788 patients with pN3a were included. In 83 patients, pN3a was based on at least an infraclavicular LNM (4.6%) and in 1705 patients because of ≥ 10 axillary LNMs (95.4%). After multivariable analyses, DFS and OS were inferior in patients with pN3a based on ≥ 10 axillary LNMs compared to infraclavicular LNM (DFS 48.8% versus 63.8%, hazard ratio [HR] 1.59, $P=0.036$; OS 46.6% versus 63.9%, HR 1.46, $P=0.042$). Furthermore, pN2a and pN3a based on infraclavicular LNM had comparable DFS and OS.

Conclusion

pN3a status based on an at least an infraclavicular LNM is rare, yet its prognosis is superior to ≥ 10 axillary LNMs. Reclassification of infraclavicular LNM in the next TNM should therefore be considered into pN2a.

Introduction

In 1958, the first edition of the tumour-node-metastasis (TNM) classification of malignant tumours of the breast was published by the Union for International Cancer Control (UICC) in order to achieve worldwide consensus for the classification of, eventually, each solid tumour type.¹ Subsequently, this classification system was revised each decade to implement new insights. For instance, the introduction of neoadjuvant systemic therapy, sentinel lymph node biopsy, immunohistochemical staining and the method of pathologic nodal staging.²

Regarding pathologic nodal staging, axillary lymph node metastases (LNMs) were divided into three categories in the fifth edition of the TNM classification: pN0 (0 axillary LNMs), pN1 (movable axillary LNMs) and pN2 (fixed axillary LNMs).³ After revision in sixth edition, the number of axillary LNMs was incorporated as key element in the classification, as impaired prognosis was demonstrated in the presence of an increasing number of axillary LNMs.⁴ This resulted in four categories: pN0 (0 axillary LNMs), pN1a (1-3 axillary LNMs), pN2a (4-9 axillary LNMs) and pN3a (≥ 10 axillary LNMs).⁵ Furthermore, a study by Newman et al. observed a worse disease-free (DFS) and overall survival (OS) in patients with infraclavicular (level III) and axillary LNMs compared to patients with axillary LNMs only (DFS 50% versus 68%; OS 58% versus 83%, respectively).⁶ As a consequence, the UICC decided to redefine infraclavicular LNM as pN3a in the sixth edition; in contrast to earlier, when an infraclavicular LNM was considered equivalent to other axillary LNMs in the fifth edition. Currently pN3a nodal status consists of patients with ≥ 10 axillary LNMs and of patients with infraclavicular LNM.⁷

The combination of both groups within pN3a suggests that their prognosis is similar.^{8,9} However, no study thus far analysed this assumption. Therefore, the purpose of this study is to determine whether the prognosis of pN3a breast cancer patients based on at least an infraclavicular LNM is different compared to patients with ≥ 10 axillary LNMs and to patients with 4-9 axillary LNMs.

Materials and methods

Data collection

Data were obtained from the Netherlands Cancer Registry (NCR), managed by the Netherlands Comprehensive Cancer Organisation (IKNL). The NCR collects data of all patients diagnosed with any type of cancer in the Netherlands, after a notification of the PALGA ('Nationwide network and registry of histo- and

cytopathology in the Netherlands') system. Afterwards, trained data collection registrars from the NCR extracted data from patients' records concerning patient characteristics, treatment and follow-up.

In this study, all patients diagnosed between 2005 and 2008 with primary invasive epithelial breast cancer and pN2a or pN3a statuses as the final pathologic result were included. Exclusion criteria were synchronous breast cancer, distant metastases at time of diagnosis (or within 91 days) or an unknown number of LNMs. Patients without surgery were also excluded. Data were collected on age, tumour type, receptor status, surgical procedures, systemic therapy, radiation therapy and pathological results, including pathologic TNM classification and the number of LNMs. For a period of 5 years after diagnosis, the first breast cancer event was registered, which consisted of any local, regional or contralateral recurrence or distant metastasis.

Patients with pN3a were divided into two subgroups according to the number of LNMs, to simulate pN3a based on infraclavicular or ≥ 10 axillary LNMs. Patients with nine or less positive lymph nodes required at least one infraclavicular LNM to be considered pN3a, while patients with ≥ 10 positive lymph nodes required at least 10 axillary LNMs (with or without infraclavicular LNMs).

Treatment

According to the national guideline of 2005, regional treatment depended on nodal status: sentinel lymph node biopsy (SLNB) in case of clinically node negative status, based on physical examination (axillary ultrasound was common but not mandatory at that time), or axillary lymph node dissection (ALND) in case of clinically node positive status, contraindication for SLNB or positive SLNB.¹⁰

Adjuvant irradiation of regional nodal fields was applied in case of four or more axillary LNMs or involvement of top axillary LNM. Recommended dose was 45-50 Gy in 5 weeks.

Systemic therapy was generally recommended for all patients with LNM. Chemotherapy was advised in all premenopausal women and in women <69 years old with estrogen (ER) and progesterone (PR) tumours. In postmenopausal women, aged 50-59 years with ER+ PR+, chemotherapy was considered in physically fit patients and in women aged 60-69 years only if four or more nodes were involved. Chemotherapy regimen consisted of five courses 5 Fluorouracil, Epirubicin, Cyclophosphamide or six courses of Taxotere, Adriamycin and Cyclophosphamide. In case of Her2Neu receptor (HER 2) amplification, targeted therapy (Trastuzumab) was given in addition to chemotherapy. Endocrine therapy was recommended for all ER+ and/or PR+ tumours.

Statistics

Statistical analyses were performed by using Statistical Package for the Social Sciences software (Version 22, IBM, Armonk, New York, USA). General characteristics between both subgroups were compared using chi-squared test for categorical data and Mann-Whitney U-test for continuous data.

For DFS, an event was defined as any local, regional or contralateral recurrence, distant metastasis or mortality within 5 years after the primary diagnosis. Events occurring 0-91 days after diagnosis were considered synchronous to the original tumour and not counted as recurrences. Date of death or date of emigration were derived from the Municipal Personal Records Database and completed until 31st December 2014. Patients were censored at the date of their first event, date of last follow-up, date of death or date of emigration, whatever came first.

DFS and OS for the pN3a subgroups, respectively, based on an infraclavicular LNM and ≥ 10 axillary LNMs, were calculated with Kaplan-Meier curves and compared with the log-rank test.¹¹ *P*-Values (two-sided) < 0.05 were considered statistically significant. Relevant clinicopathological variables associated with DFS and OS were examined using univariable and, where applicable, multivariable Cox proportional hazards regression, with hazard ratio (HR) and corresponding 95% confidence intervals. The number of variables used for multivariable Cox proportional hazards regression depends on the number of outcome events per predictor variable, which requires at least five events per variable.¹²

Finally, DFS and OS of patients with pN3a based on infraclavicular LNM were compared to patients with pN2a (i.e. 4-9 axillary LNMs), by calculating Kaplan-Meier curves and comparing with the log-rank test. In addition, univariable and multivariable Cox proportional hazards regression evaluated association of relevant clinicopathological variables associated with DFS and OS.

Results

A total of 51,239 patients were diagnosed with primary invasive epithelial breast cancer between 2005 and 2008 in the Netherlands, of whom 3442 patients had pN2a (6.6%) and 1799 patients (3.5%) had pN3a status (Figure 7.1). Eventually, 83 patients were classified as pN3a based on infraclavicular LNM (4.6%) and 1705 patients based on ≥ 10 axillary LNMs (95.4%). Compared to patients with ≥ 10 axillary LNMs, patients with at least an infraclavicular LNM were younger (55 versus 59 years, $P=0.010$), less often had pT3-4 tumours (15% versus 24%, $P=0.049$) with a smaller mean tumour size (31 versus 36 mm, $P=0.032$) and, obviously, had fewer positive lymph nodes (mean 6 versus 15, $P<0.001$). A more detailed overview of the general characteristics is provided in Table 7.1.

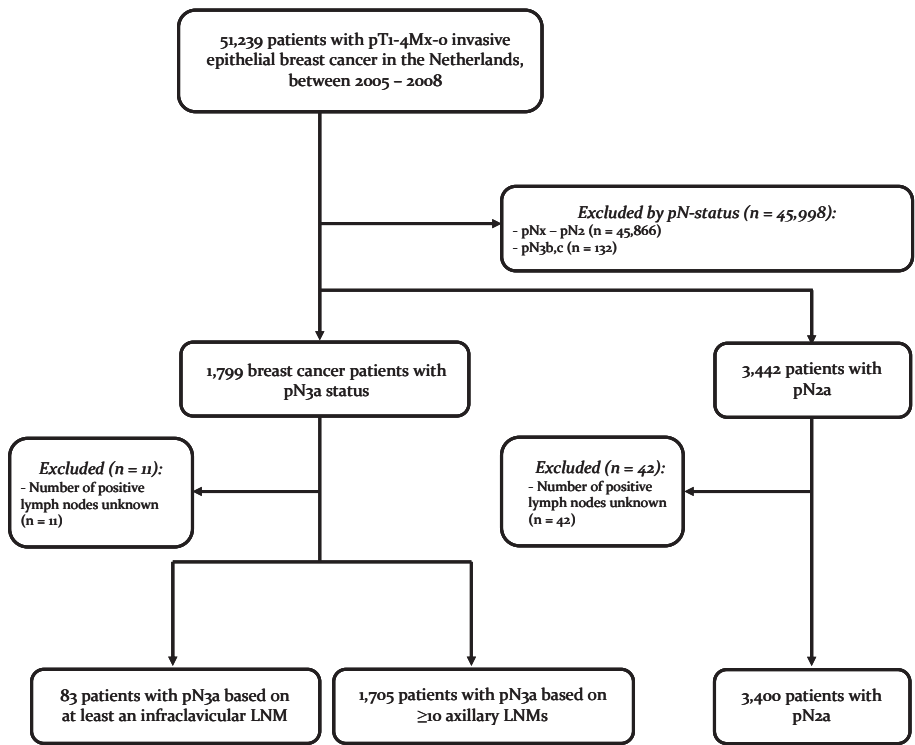


Figure 7.1 Flowchart of included patients. Abbreviations: *pN* pathologic nodal status, *LNM* lymph node metastases.

Table 7.1 General patient and tumour characteristics.

	pN3a Infraclavicular LNM (n=83)	pN3a ≥10 axillary LNMs (n=1705)	P-value
Mean age (years) (range)	55.3 (30 - 84)	59.1 (26 - 97)	0.010
Pathologic T-stage (%)			
T0-2	69 (83.1)	1252 (73.4)	0.049
T3-4	12 (14.5)	406 (23.8)	0.049
Unknown	2 (2.4)	47 (2.8)	0.850
Mean tumour size (mm) (range)	31.4 (6 - 114)	35.6 (0 - 250)	0.032
Tumour grade (%)			
1-2	39 (47.0)	700 (41.1)	0.284
3	33 (39.7)	785 (46.0)	0.262
Unknown	11 (13.3)	220 (12.9)	0.926
Mean number of positive lymph nodes (range)	5.7 (1 - 9)	15.2 (10 - 53)	<0.001
ER (%)			
Positive	64 (77.1)	1244 (73.0)	0.405
Negative	19 (22.9)	451 (26.4)	0.472
Unknown	0	10 (0.6)	1.000

Table 7.1 (continued)

	pN3a Infracavicular LNM (n=83)	pN3a ≥10 axillary LNMs (n=1705)	P-value
PR (%)			
Positive	48 (57.8)	896 (52.6)	0.347
Negative	34 (41.0)	723 (42.4)	0.795
Unknown	1 (1.2)	86 (5.0)	0.183
Her2 (%)			
Positive	12 (14.5)	346 (20.3)	0.195
Negative	65 (78.3)	1247 (73.1)	0.298
Equivocal	3 (3.6)	59 (3.5)	0.763
Unknown	3 (3.6)	53 (3.1)	0.743
Tumour type (%)			
Invasive carcinoma NST	58 (69.9)	1151 (67.5)	0.652
Lobular	15 (18.1)	344 (20.2)	0.640
Mixed ductal and lobular	5 (6.0)	92 (5.4)	0.805
Other	5 (6.0)	118 (6.9)	0.753
Subtype (%)			
ER+PR+, Her2-	41 (49.4)	747 (43.8)	0.224
ER+PR-, Her2-	13 (15.7)	241 (14.1)	0.697
ER+Her2+	5 (6.0)	168 (9.9)	0.249
ER-Her2+	7 (8.4)	174 (10.2)	0.601
Triple negative	12 (14.5)	238 (14.0)	0.898
Unknown	4 (4.8)	127 (7.4)	0.369
Breast surgery (%)			
Breast conserving therapy	22 (26.5)	339 (19.9)	0.142
Mastectomy	61 (73.5)	1363 (79.9)	0.159
Unknown	0	3 (0.2)	1.000
Axillary surgery (%)			
SLNB	4 (4.8)	2 (0.1)	<0.001
SLNB followed by ALND	26 (31.3)	316 (18.6)	0.004
ALND	53 (63.9)	1378 (80.8)	<0.001
Unknown	0	9 (0.5)	1.000
Radiation therapy (%)			
Yes	76 (91.6)	1528 (89.6)	0.569
Chemotherapy (%)			
Yes	67 (80.7)	1267 (74.3)	0.190
Endocrine therapy to ER+ subtype (%)			
Yes	58 (90.6)	1130 (90.8)	0.955
Trastuzumab to Her2+ subtype (%)			
Yes	8 (66.7)	254 (73.4)	0.604

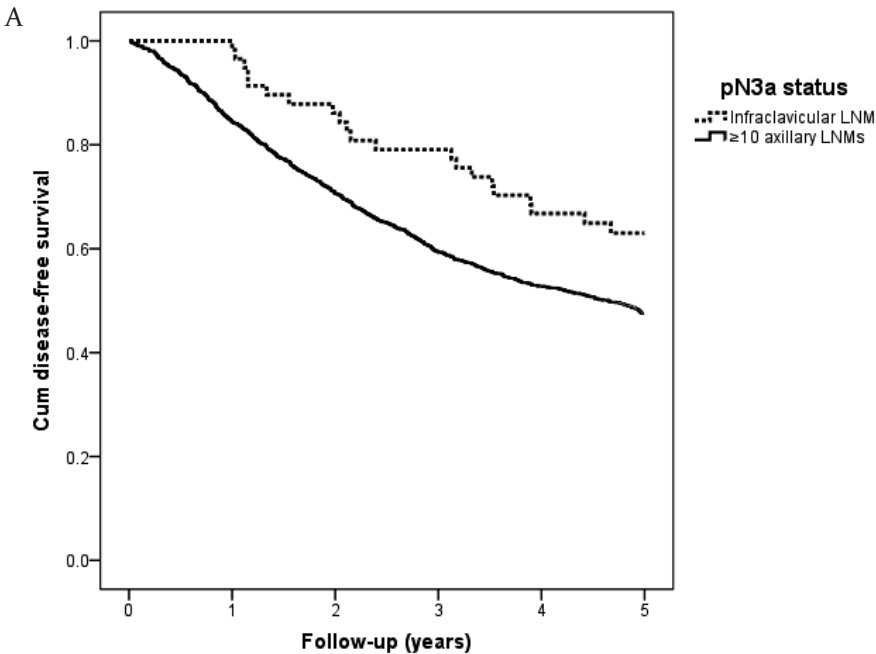
Abbreviations: ER estrogen, PR progesteron, Her2 human epidermal growth factor receptor 2, SLNB sentinel lymph node biopsy, ALND axillary lymph node dissection.

Disease-free survival

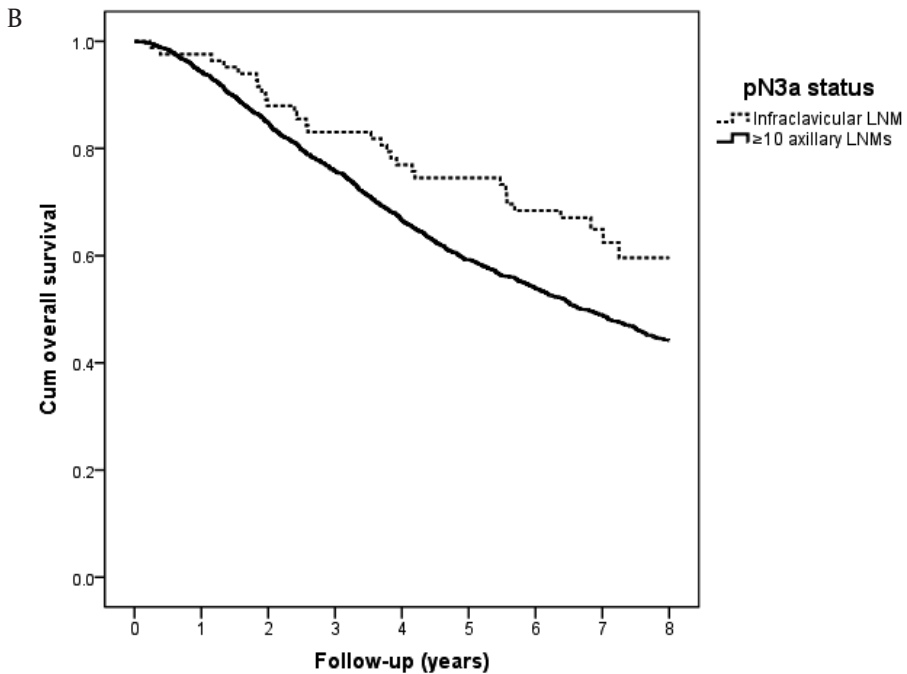
Five year follow-up was available for 1293 patients (72.3%, n=58 versus n=1235, for patients with at least an infracavicular versus ≥10 axillary LNMs). Within 5 years after diagnosis, 43.6% experienced a first locoregional or contralateral recurrence

or distance metastasis and 6.9% deceased. Thus 50.5% of the patients experienced an event, resulting in a DFS of 49.5%. In subgroup analyses, DFS was 63.8% in patients with at least an infraclavicular LNM and 48.8% of patients with ≥ 10 axillary LNMs ($P=0.018$) (Figure 7.2a).

In multivariable Cox regression analyses, the effect of having ≥ 10 axillary LNMs on DFS was significant (HR 1.59, $P=0.036$) (Table 7.2). Receiving chemotherapy (HR 0.51, $P<0.001$) and radiation therapy (HR 0.59, $P<0.001$) were identified as significant predictors for increased DFS, whereas triple negative subtype (HR 2.57, $P<0.001$) was identified as significant predictor for decreased DFS.



Infraclavicular LNM	58	57	49	45	37	23
≥ 10 axillary LNMs	1235	1040	865	721	625	356



Infraclavicular LNM	83	81	73	68	63	61	56	26	12
≥10 axillary LNMs	1705	1607	1444	1288	1130	1004	912	627	373

Figure 7.2 (A) and (B) Kaplan-Meier curves for disease-free survival after 5 years of follow up and overall survival after 8 years, including the number of patients at risk. LNM, lymph node metastases.

Overall survival

After 8 years of follow-up, 47.4% of all patients were alive. This concerned 63.9% of patients with at least an infraclavicular LNM and 46.6% with ≥ 10 axillary LNMs ($P=0.009$) (Figure 7.2b).

In multivariable Cox regression analyses, the effect of having ≥ 10 axillary LNMs on OS was statistically significant (HR 1.46, $P=0.042$) (Table 7.3). Significant predictors for decreased OS were the presence of pT3-4 tumours (HR 1.60, $P<0.001$) and triple negative subtype (HR 1.79, $P<0.001$). Receiving chemotherapy (HR 0.42, $P<0.001$), endocrine therapy (HR 0.60, $P<0.001$) and radiation therapy (HR 0.53, $P<0.001$) were identified as significant predictors for increased OS.

Comparison of infraclavicular LNM to pN2a nodal status

In the subgroup of pN2a, 5-year follow-up was available for 2483 patients (73.0%) with a DFS of 67.3%. Compared to patients with pN3a based on infraclavicular LNM (DFS 63.8%) this was not statistically significant ($P=0.661$) (Appendix 7.1a). In multivariable Cox regression analyses, the effect of having pN3a based on infraclavicular LNM on DFS remained comparable to pN2a (HR 1.17, $P=0.491$) (Appendix 7.2a).

After 8 years of follow-up, 65.5% of pN2a patients were alive. Again, which was not different compared to OS of patients with pN3a based on infraclavicular LNM (OS 63.9%) ($P=0.500$) (Appendix 7.1b). In multivariable Cox regression analyses, the effect of having pN3a based on infraclavicular LNM on OS remained comparable to pN2a (HR 1.25, $P=0.233$) (Appendix 7.2b).

Table 7.2 Uni- and multivariable analyses of predictors for disease-free survival.

	Univariable analysis		Multivariable analysis	
	HR (95%CI)	P-value	HR (95%CI)	P-value
Infraclavicular LNM	Reference		Reference	
≥10 axillary LNMs	1.68 (1.09 – 2.59)	0.020	1.59 (1.03 – 2.46)	0.036
Age (per year increment)	1.02 (1.02 – 1.03)	<0.001	-	-
pT-stage T3-4 vs. T0-2	1.65 (1.39 – 1.96)	<0.001	-	-
Tumour grade 3 vs. 1-2	1.45 (1.24 – 1.69)	<0.001	-	-
Triple negative subtype Yes vs. No	2.43 (2.01 – 2.94)	<0.001	2.57 (2.13 – 3.11)	<0.001
Chemotherapy Yes vs. No	0.49 (0.42 – 0.58)	<0.001	0.51 (0.43 – 0.60)	<0.001
Trastuzumab Yes vs. No	0.74 (0.59 – 0.92)	0.007	-	-
Endocrine therapy Yes vs. No	0.50 (0.43 – 0.59)	<0.001	-	-
Radiation therapy Yes vs. No	0.48 (0.37 – 0.60)	<0.001	0.59 (0.46 – 0.75)	<0.001

Abbreviations: LNM lymph node metastases, pT-stage pathologic tumour stage.

Table 7.3 Uni- and multivariable analyses of predictors for overall survival.

	Univariable analysis		Multivariable analysis	
	HR (95%CI)	P-value	HR (95%CI)	P-value
Infraclavicular LNM	Reference		Reference	
≥10 axillary LNMs	1.61 (1.12 – 2.32)	0.010	1.46 (1.01 – 2.10)	0.042
Age (per year increment)	1.03 (1.03 – 1.04)	<0.001	-	-
pT-stage T3-4 vs T0-2	1.56 (1.35 – 1.79)	<0.001	1.60 (1.39 – 1.85)	<0.001
Tumour grade 3 vs 1-2	1.43 (1.26 – 1.63)	<0.001	-	-
Triple negative subtype Yes vs No	2.38 (2.03 – 2.80)	<0.001	1.79 (1.47 – 2.19)	<0.001
Chemotherapy Yes vs No	0.42 (0.36 – 0.48)	<0.001	0.42 (0.36 – 0.48)	<0.001
Trastuzumab Yes vs No	0.64 (0.53 – 0.78)	<0.001	-	-
Endocrine therapy Yes vs No	0.51 (0.45 – 0.58)	<0.001	0.60 (0.51 – 0.71)	<0.001
Radiation therapy Yes vs No	0.38 (0.32 – 0.45)	<0.001	0.53 (0.44 – 0.64)	<0.001

Abbreviations: LNM lymph node metastases, pT-stage pathologic tumour stage.

Discussion

According to the sixth and seventh edition of the TNM classification for breast cancer, pathologic nodal status is defined using the number and location of LNMs. A pN3a status in breast cancer consists either of at least an infraclavicular (level III) or ≥ 10 axillary LNMs.⁷ Inclusion of both groups in the same category of TNM suggests a similar prognosis.^{8,9,13} However, our study demonstrated superior DFS and OS in patients with pN3a based on at least an infraclavicular LNM compared to ≥ 10 axillary LNMs. Furthermore, DFS and OS of patients with pN3a based on at least an infraclavicular LNM compared to patients with pN2a were comparable according to our study.

The decision to redefine infraclavicular LNM as pN3a breast cancer was solely based on the results of a study of Newman et al..^{2,6} In this study, incidence and prognosis of infraclavicular LNM among patients with axillary LNMs was investigated, which showed worse DFS and OS in case of suspicious adenopathy.⁶ Study limitations were the absence of pathological confirmation of the suspicious nodes, as well as potentially confounding factors like presence of supraclavicular LNM in some patients.¹⁴ In our study, pN3a was defined according to final pathological report, resulting in pathologically confirmed infraclavicular LNM in all 83 patients and therefore representing a more valid patient population.

Classification of infraclavicular LNM as pN3a disregards the number of nodal metastases and the size of the largest metastasis. A single micrometastasis in an infraclavicular lymph node would represent pN3a status, whereas pN3a without infraclavicular involvement would require ≥ 10 LNMs with at least one macrometastasis.⁷ Disregarding size of the nodal metastases by only taking infraclavicular location into account can explain part of the difference in DFS and OS between pN3a based on at least an infraclavicular LNM and ≥ 10 axillary LNMs.

DFS after 5 years in patients with ≥ 10 axillary LNMs in our study cohort is comparable to previous results of Koca et al., in which 5-year DFS was 46.2% in patients with ≥ 10 axillary LNMs.¹⁵ In a similar cohort of patients with ≥ 10 axillary LNMs, Turker et al. demonstrated the highest 5-year DFS rate of 49.2% in patients with ER/PR+ and Her2-subtype.¹⁶ These results confirm our findings concerning DFS in patients with pN3a based on ≥ 10 axillary LNMs, which was 48.8%.

The definition of an infraclavicular (level III) lymph node during surgery may be open to interpretation. According to the American Society of Breast Surgeons, a level III ALND (extending to the apex of the axilla) is only recommended in patients with evidence of suspicious nodes located behind the pectoralis minor muscle (level II).¹⁷ However, in some cases a suspicious level II node can be incorrectly defined as infraclavicular (level III) node during ALND. As a consequence, these patients were considered pN3a based on infraclavicular LNM

rather than potentially pN2a or pN1a, depending on the total number of axillary LNMs.

Due to new imaging techniques, the detection of infraclavicular LNM has increased over time. Prior to the introduction of the sixth edition of TNM in 2002, infraclavicular LNMs were detected during physical examination and/or surgery. In the current era, with imaging modalities like breast magnetic resonance imaging (MRI) and positron-emission tomography/computed tomography (PET/CT), smaller (infraclavicular) LNMs can be detected prior to surgery.^{18,19} However, the seventh edition of TNM is still based on a 2001 study in which infraclavicular LNMs were detected with physical examination and ultrasound rather than MRI or PET-CT.^{2,6} Our cohort consisted of patients diagnosed between 2005 and 2008, which is more in line with the current imaging modalities. MRI was already recommended in our study cohort according to the national guidelines of 2005.^{7,10}

Although the incidence of patients with pN3a based on at least an infraclavicular LNM in our cohort is small (4.6%), our findings suggest that reclassification in the next TNM classification should be considered. We advise to redefine an infraclavicular LNM as equivalent to other axillary LNMs rather than taking the location of infraclavicular LNM into account. Consequently, patients with an infraclavicular LNM with ≤ 9 LNMs will be considered pN2a rather than pN3a. In this way, infraclavicular LNM will become consistent with intramammary and interpectoral LNM, which are coded as axillary LNMs (level I/II) in the current TNM classification.⁷ Yet, adjuvant (radiation) therapy of infraclavicular LNM is still recommended.

This study had limitations. A major limitation of this study concerns the subgroup of patients with ≥ 10 axillary LNMs, which still might have infraclavicular LNM as well. Yet, the focus of this study was to compare prognosis between both subgroups, since the current TNM atlas considers both as one category. Our results should therefore be interpreted with this important limitation in mind.

A second limitation of this study was the use of a retrospective database. Some clinically relevant parameters were not present, for instance, radiation therapy fields and the presence of lymphovascular invasion of the tumour. As a consequence, irradiation of regional nodal fields is unknown in this study cohort, which is generally recommended in breast cancer patients with LNMs.²⁰ Furthermore, presence of lymphovascular invasion can have a negative effect on overall survival.²¹

Third, these data only contain patients treated in the Netherlands between 2005 and 2008. This might have influence on prognosis when these data would be extrapolated to cohorts in other countries. For instance, the 5-year survival rate of breast cancer patients in the Netherlands still is different when compared to Asian or South American countries.²²

Fourth, despite the collection of nationwide data between 2005 and 2008 in the Netherlands, the subgroup of patients with pN3a based on at least an infraclavicular LNM remained small (n=83). As a consequence, the number of variables for multivariable Cox proportional hazards regression was restricted due to the limited number of events.¹² However, differences between both subgroups regarding tumour subtypes and adjuvant treatment were small, which means that it is unlikely that the difference in prognosis would be attributable to the difference in covariates between the two cohorts.

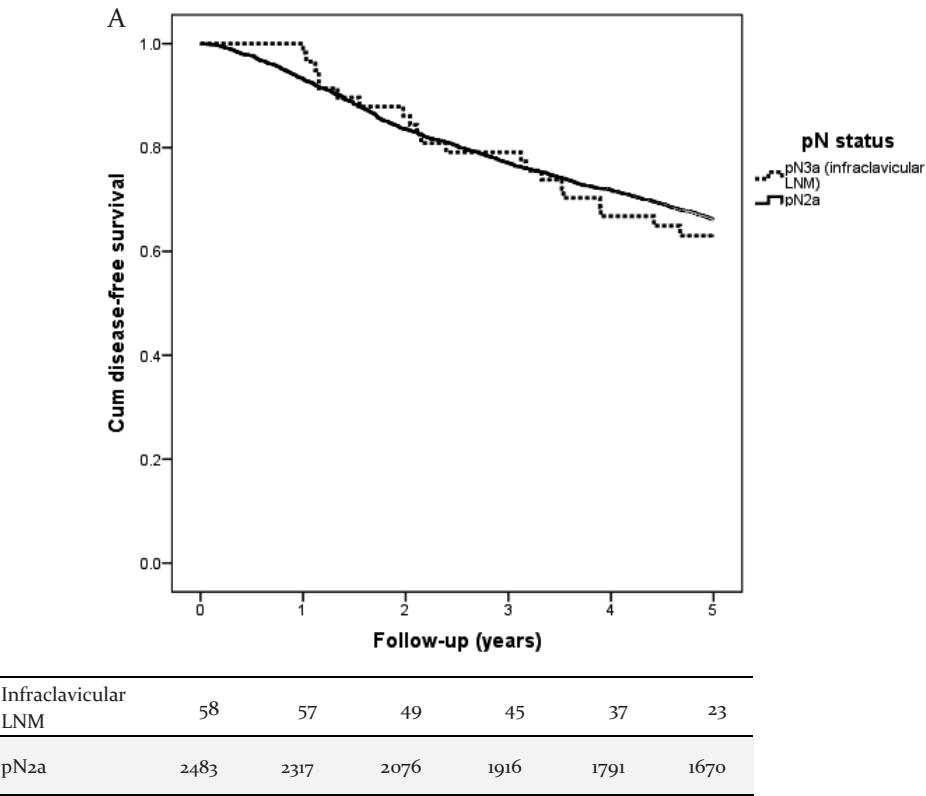
In conclusion, DFS and OS of patients staged as pN3a based on at least an infraclavicular LNM is superior compared to patients with ≥ 10 axillary LNMs. Therefore, reclassification of infraclavicular LNM in the next edition of TNM should be considered to classify an infraclavicular LNM with fewer than 10 LNMs to pN2a rather than pN3a.

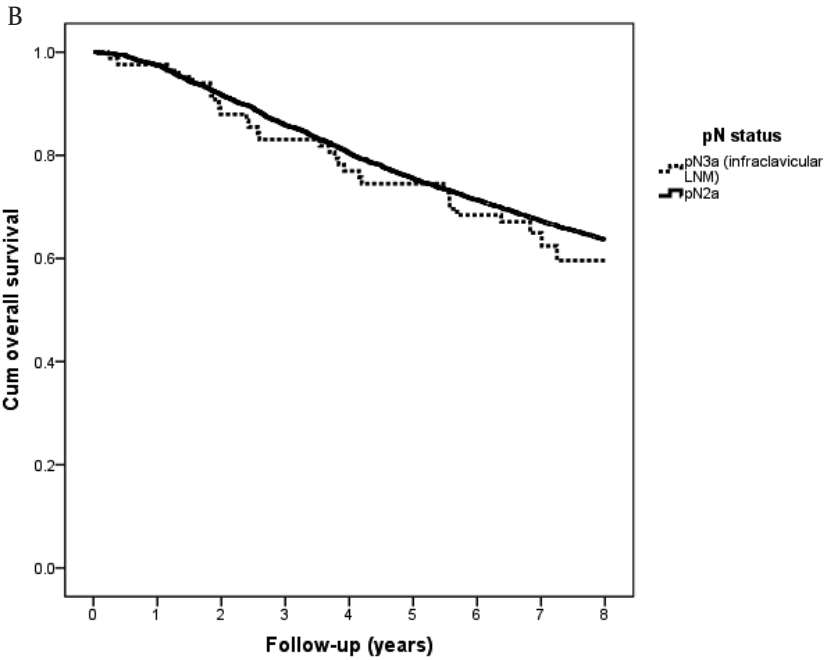
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Appendix 7.1





Intraclavicular LNM	83	81	73	68	63	61	56	26	12
pN2a	1705	1607	1444	1288	1130	1004	912	627	373

Appendix 7.2

A

	Univariable analysis		Multivariable analysis	
	HR (95%CI)	P-value	HR (95%CI)	P-value
pN2a	Reference		Reference	
Infraclavicular LNM	1.10 (0.71 – 1.70)	0.661	1.17 (0.76 – 1.80)	0.491
Age (per year increment)	1.03 (1.02 – 1.03)	<0.001	-	-
pT-stage T3-4 vs To-2	1.94 (1.64 – 2.31)	<0.001	-	-
Tumour grade 3 vs 1-2	1.65 (1.44 – 1.88)	<0.001	-	-
Triple negative subtype Yes vs No	2.92 (2.47 – 3.46)	<0.001	3.08 (2.60 – 3.65)	<0.001
Chemotherapy Yes vs No	0.41 (0.35 – 0.47)	<0.001	0.44 (0.38 – 0.51)	<0.001
Trastuzumab Yes vs No	0.72 (0.59 – 0.88)	0.001	-	-
Endocrine therapy Yes vs No	0.42 (0.36 – 0.48)	<0.001	-	-
Radiation therapy Yes vs No	0.40 (0.33 – 0.49)	<0.001	0.52 (0.43 – 0.64)	<0.001

Abbreviations: LNM lymph node metastases, pT-stage pathologic tumour stage.

B

	Univariable analysis		Multivariable analysis	
	HR (95%CI)	P-value	HR (95%CI)	P-value
pN2a	Reference		Reference	
Infraclavicular LNM	1.13 (0.79 – 1.63)	0.501	1.25 (0.87 – 1.79)	0.233
Age (per year increment)	1.04 (1.04 – 1.05)	<0.001	-	-
pT-stage T3-4 vs To-2	1.88 (1.64 – 2.18)	<0.001	1.82 (1.58 – 2.10)	<0.001
Tumour grade 3 vs 1-2	1.50 (1.34 – 1.68)	<0.001	-	-
Triple negative subtype Yes vs No	2.68 (2.32 – 3.10)	<0.001	1.75 (1.47 – 2.10)	<0.001
Chemotherapy Yes vs No	0.31 (0.28 – 0.35)	<0.001	0.34 (0.30 – 0.38)	<0.001
Trastuzumab Yes vs No	0.62 (0.52 – 0.74)	<0.001	-	-
Endocrine therapy Yes vs No	0.44 (0.39 – 0.49)	<0.001	0.51 (0.45 – 0.59)	<0.001
Radiation therapy Yes vs No	0.34 (0.30 – 0.40)	<0.001	0.56 (0.48 – 0.65)	<0.001

Abbreviations: LNM lymph node metastases, pT-stage pathologic tumour stage.

Chapter 8

Prognosis of residual axillary disease after neoadjuvant chemotherapy in clinically node-positive breast cancer patients: isolated tumour cells and micrometastases carry a better prognosis than macrometastases

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Abstract

Purpose

The aim of this study was to compare disease-free survival (DFS) and overall survival (OS) between clinically node-positive breast cancer patients, treated with neoadjuvant chemotherapy (NAC), with axillary pathologic complete response (ypNo), residual axillary isolated tumour cells or micrometastases (ypN1c/mi), and residual axillary macrometastases (ypN1-3).

Methods

All patients diagnosed with clinically node-positive primary invasive breast cancer treated with NAC and subsequent axillary lymph node dissection between 2005 and 2008 were retrospectively analyzed. Data were obtained from the Netherlands Cancer Registry. Patients were stratified by final pathological axillary status: ypNo, ypN1c/mi, or ypN1-3. The main outcome measures DFS and OS were analyzed using Kaplan–Meier survival analysis. Uni- and multivariable cox regression analyses were used to determine independent predictors for DFS and OS.

Results

A total of 1347 patients were included. Pathologic nodal status was ypNo in 22.2%, ypN1c/mi in 3.8%, and ypN1-3 in 74.0% of patients. Overall, 5-year DFS was 57.8% and mean OS was 7.4 years. DFS and OS were comparable between ypNo and ypN1c/mi (HR 1.38 (0.40–4.79, $P=0.613$) and HR 0.92 (0.27–3.09, $P=0.889$), respectively), but significantly different between ypNo and ypN1-3 (HR 1.78 (1.06–3.00, $P=0.031$) and HR 1.70 (1.07–2.71, $P=0.026$), respectively).

Conclusions

Clinically node-positive patients, treated with NAC, with axillary nodal status ypNo or ypN1c/mi carry similar prognosis regarding DFS and OS. Axillary nodal status ypN1-3 is associated with a less favourable prognosis. Future studies should consider ypNo and ypN1c/mi as one entity.

Introduction

Over the past 20 years, a trend toward a less invasive approach regarding the surgical management of the axilla in breast cancer patients has been observed. Nowadays, a sentinel lymph node biopsy (SLNB) has been widely adopted for staging of early-stage clinically node-negative breast cancer.¹ In case of a sentinel lymph node (SLN) containing isolated tumour cells (ITCs) or micrometastases, a completion of axillary lymph node dissection (ALND) does not improve survival, nor does it reduce regional recurrence. Consequently, ALND following SLNB has been abandoned in these patients.²⁻⁴ The ACOSOG Z0011 trial demonstrated no significant effect on prognosis when ALND is omitted in case of a SLN containing a limited number of metastases, even macrometastases, in patients treated with breast conserving therapy.³

In clinically node-positive (cN+) patients, ALND is regarded as standard surgical therapy. However, increased utilization of neoadjuvant chemotherapy (NAC) results in axillary pathologic complete response (pCR) in 30-40% of patients.⁵ Consequently, the value of ALND is topic of debate. Various studies demonstrated that axillary pCR after NAC is associated with improved prognosis.⁶⁻⁸ Residual axillary disease has a less favourable prognosis, but it is unknown whether different degrees of residual axillary disease (i.e., ITCs, micrometastases, macrometastases) all have similar prognosis.

Hence, the purpose of this study was to compare prognosis of axillary pCR, residual ITCs, or micrometastases and residual macrometastases in cN+ patients treated with NAC.

Methods

Data collection

In this study, all pathologically confirmed cN+ patients diagnosed with primary invasive breast cancer and treated with NAC (with or without immunotherapy) followed by ALND between 2005 and 2008 were included. Exclusion criteria were synchronous breast cancer, primary surgical treatment, neoadjuvant radiation therapy, neoadjuvant endocrine therapy, unknown pathological nodal status, and distant metastases diagnosed within 91 days after primary breast cancer diagnosis. Patients who did not undergo ALND were also excluded.

Data were obtained from the Netherlands Cancer Registry (NCR), which is managed by the Netherlands Comprehensive Cancer Organisation (NCCO). The PALGA foundation (Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief), a nationwide network and registry of histopathology and cytopathology

diagnosis in the Netherlands, regularly submits reports of all diagnosed malignancies to the cancer registry. After notification, trained data collection registrars from the NCR extracted data from patients' records. Data were collected on age, tumour type, receptor status, surgical procedures, systemic therapy, adjuvant radiation therapy, and pathology results, including pathological TNM stage and tumour grade. During a 5-year period after initial diagnosis, the first of the following breast cancer events was registered: any local, regional, or contralateral recurrence or distant metastasis. Date of death or date of emigration was derived from the Municipal Personal Records Database (Basisregistratie Personen, BRP) and files until December 31, 2014 were analyzed. Patients were stratified into three subgroups according to final pathologic axillary nodal status after completion of NAC and definitive surgery: pCR (ypNo), residual isolated tumour cells or micrometastases (ypN1c/mi), and residual macrometastases (ypN1-3).

Neoadjuvant chemotherapy (NAC) with/without immunotherapy regimen

During the study period, the Dutch national guideline of 2005 was in use.⁹ This guideline recommended chemotherapy regimens consisting of five courses 5 Fluorouracil, Epirubicin, Cyclophosphamide (FEC), or six courses of Taxotere, Adriamycin, and Cyclophosphamide (TAC). In case of Her2Neu receptor (Her2) amplification, targeted therapy (trastuzumab) was recommended in addition to chemotherapy.

Statistics

Statistical analyses were performed using Statistical Package for the Social Sciences software (Version 22, IBM, Armonk, New York, USA). General characteristics between the three subgroups were compared using Chi squared test for categorical data and One-way ANOVA for continuous data, after confirmation of Levene's test for equality of variances. If Levene's test demonstrated significant differences among the population variances, Kruskal-Wallis test was used.

DFS was defined as time from diagnosis to any local (including carcinoma in situ), regional, or contralateral recurrence, distant metastasis or mortality within 5 years after the primary diagnosis. Events occurring 0–91 days after diagnosis were considered synchronous to the original tumour and were not counted as recurrence. OS was defined as the time interval between date of diagnosis and date of death, date of first event, date of last follow-up, or date of emigration.

DFS and OS for the three subgroups were calculated with Kaplan-Meier curves and compared with the log-rank test. *P* values (two-sided) <0.05 were

considered statistically significant. Relevant clinicopathological variables associated with DFS and OS were examined using univariable and, where applicable, multivariable Cox proportional hazards regression, with Hazard Ratio (HR) and corresponding 95% confidence intervals.

Results

Between 2005 and 2008, 8176 patients were diagnosed with cN+ breast cancer in the Netherlands. Patients were excluded for several reasons: 6553 patients underwent primary surgery; 204 patients did not undergo ALND; 9 patients were treated with neoadjuvant radiotherapy; 61 patients were treated with neoadjuvant endocrine therapy; and ypN status was unknown for 11 patients (Figure 8.1). A final total of 1347 patients were included for this study: 299 ypNo, 51 ypN1c/mi and 997 ypN1-3.

The incidence of pCR of the primary tumour was higher in patients with ypNo compared to ypN1c/mi and ypN1-3 patients (41.1% vs. 19.6% and 7.1%, respectively, $P<0.001$). Furthermore, lobular carcinoma was observed more often in patients with ypN1-3 than in ypNo and ypN1c/mi patients (9.4% vs. 5.4% and 3.9%, respectively, $P=0.039$). Adjuvant radiation therapy was applied more often in ypN1-3 as compared to ypNo and ypN1c/mi patients (92.1% vs. 80.9% and 80.4%, respectively, $P<0.001$, Table 8.1).

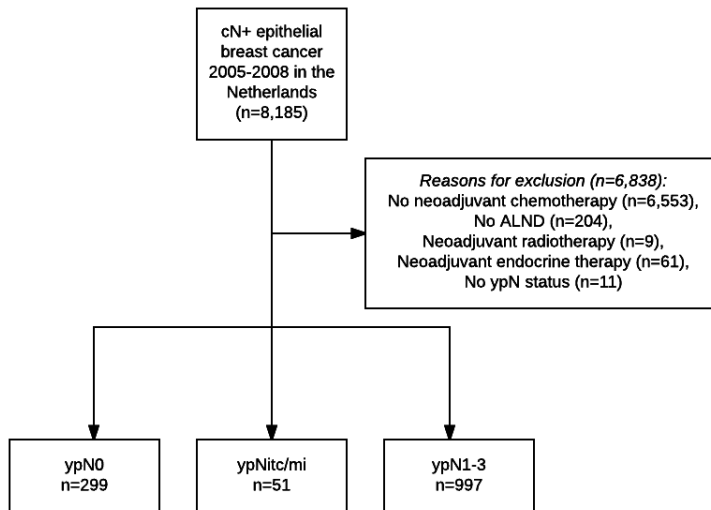


Figure 8.1 Flowchart of included patients. Abbreviations: cN+ clinically node positive status, SLNB sentinel lymph node biopsy, ALND axillary lymph node dissection, ypNo axillary pathologic complete response, ypN1c/mi axillary residual isolated tumour cells or micrometastases, ypN1-3 axillary residual macrometastases.

Table 8.1 General characteristics.

	<i>ypNo</i> (<i>n</i> =299)	<i>ypN1tc/mi</i> (<i>n</i> =51)	<i>ypN1-3</i> (<i>n</i> =997)	<i>P</i> -value
Mean age (years) (range)	48.9 (27 – 77)	48.2 (29 – 81)	50.4 (22 – 85)	0.053
Clinical T-stage (%)				
cT0-is	1 (0.3)	0	5 (0.5)	0.826
cT1-2	138 (47.0)	26 (52.0)	458 (47.0)	0.780
cT3-4	153 (52.0)	24 (48.0)	509 (52.0)	0.853
cTx	7	1	25	-
Pathologic T-stage (%)				
ypT0-is	123 (50.8)	10 (26.3)	71 (8.5)	<0.001
ypT1-2	107 (44.2)	25 (65.8)	570 (68.5)	<0.001
ypT3-4	12 (5.0)	3 (7.9)	191 (23.0)	<0.001
Unknown	57	13	165	-
Tumour grade (%)				
1-2	28 (31.5)	7 (50.0)	197 (43.9)	<0.001
3	61 (68.5)	7 (50.0)	252 (56.1)	0.051
Unknown	210	37	548	-
Tumour type (%)				
Ductal	227 (75.9)	41 (80.4)	746 (74.8)	0.739
Lobular	16 (5.4)	2 (3.9)	94 (9.4)	0.039
Other ^a	56 (18.7)	8 (15.7)	157 (15.8)	0.470
Subtype (%)				
ER+PR+, Her2-	35 (12.8)	11 (22.5)	349 (37.3)	<0.001
ER+PR-, Her2-	21 (7.7)	7 (14.3)	122 (13.0)	0.035
ER+Her2+	47 (17.1)	20 (40.8)	152 (16.3)	<0.001
ER-Her2+	97 (35.4)	5 (10.2)	146 (15.6)	<0.001
Triple negative	74 (27.0)	6 (12.2)	166 (17.8)	0.003
Unknown	25	2	62	-
Breast surgery (%)				
Breast conserving therapy	62 (20.7)	12 (23.5)	181 (18.2)	0.421
Mastectomy	237 (79.3)	39 (76.5)	816 (81.8)	0.421
Unknown	0	0	1	-
Radiation therapy (%)				
Yes	242 (80.9)	41 (80.4)	918 (92.1)	<0.001
Endocrine therapy to ER+ subtype (%)				
Yes	95 (84.8)	35 (92.1)	600 (91.5)	0.080
Trastuzumab to Her2+ subtype (%)				
Yes	127 (92.0)	19 (76.0)	247 (89.2)	0.057

^a Including adenocarcinoma not otherwise specified, mucinous carcinoma, and mixed carcinoma.

Abbreviations: *ypNo* axillary pathologic complete response, *ypN1tc/mi* axillary residual isolated tumour cells or micrometastases, *ypN1-3* axillary residual macrometastases, *cT-stage* clinical tumour stage, *pT-stage* pathologic tumour stage, *ER* estrogen, *PR* progesterone, *Her2* human epidermal growth factor receptor 2.

Disease-free survival

Five-year follow-up was available for 944 patients (70.1%; *n*=206 *ypNo*, *n*=34 *ypNoi*+/*ypN1mi*, *n*=704 *ypN1-3*): Recurrence occurred in 377 patients (39.9%) and 22 patients died within 5 years (2.3%). This resulted in a DFS event in 42.2% of the

patients. DFS did not differ significantly between ypNo and ypN1tc/mi (71.8 vs. 70.6%, $P=0.978$). When DFS was compared between ypNo and ypN1-3, a significant difference was found (71.8 vs. 53.4%; $P=0.049$) (Figure 8.2a).

Multivariable Cox regression analyses demonstrated no significant difference in DFS between ypNo and ypN1tc/mi (HR 1.38 (0.40–4.79), $P=0.613$), but a significant difference in DFS between ypNo and ypN1-3 (HR 1.78 (1.06–3.00), $P=0.031$) (Table 8.2).

Furthermore, higher ypT stage (ypT 1-2: HR 2.73 (1.39–5.39), $P=0.004$ and ypT 3-4: HR 4.71 (2.35–9.43), $P<0.001$) and higher tumour grade (HR 1.69 (1.19–2.40), $P=0.004$) were identified as independent predictors of decreased DFS, whereas endocrine therapy was identified as independent predictor of increased DFS (HR 0.55 (0.36–0.85), $P=0.007$).

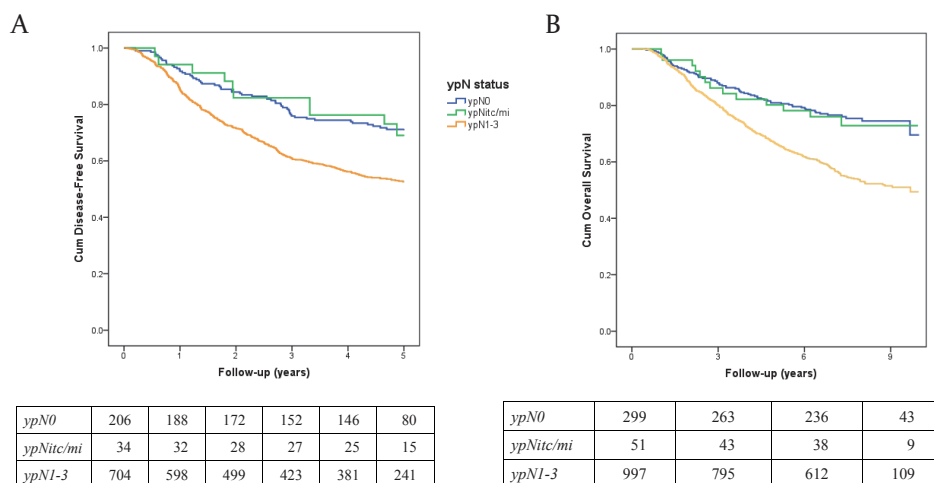


Figure 8.2 Kaplan–Meier curves for disease-free (A) and overall survival (B), including number at risk. Abbreviations: *ypN status* pathologic nodal status after neoadjuvant chemotherapy, *ypN0* axillary pathologic complete response, *ypN1tc/mi* axillary residual isolated tumour cells or micrometastases, *ypN1-3* axillary residual macrometastases.

Overall survival

Mean OS was 7.4 years (range 0.4–10 years): 8.3 years for ypNo, 8.2 years for ypN1tc/mi, and 7.0 years for ypN1-3 (Figure 8.2b). OS was comparable between ypNo and ypN1tc/mi ($P=0.875$). However, OS was significantly lower for ypN1-3 as compared to ypNo ($P=0.014$).

Table 8.2 Uni- and multivariable analyses of predictors for disease-free survival at 5 years.

	Univariable analysis		Multivariable analysis	
	HR (95%CI)	P-value	HR (95%CI)	P-value
ypNo	Reference		Reference	
ypN1c/mi	1.02 (0.52 – 1.99)	0.964	1.38 (0.40 – 4.79)	0.613
ypN1-3	1.89 (1.43 – 2.50)	<0.001	1.78 (1.06 – 3.00)	0.031
Age (per year increment)	1.02 (1.01 – 1.02)	0.001	1.01 (0.99 – 1.02)	0.479
ypT-stage				
To or Tis	Reference		Reference	
T1-2	1.88 (1.31 – 2.70)	0.001	2.73 (1.39 – 5.39)	0.004
T3-4	3.74 (2.53 – 5.54)	<0.001	4.71 (2.35 – 9.43)	<0.001
Tumour type				
Ductal	Reference		Reference	
Lobular	0.78 (0.53 – 1.14)	0.193	1.11 (0.59 – 2.07)	0.751
Other	0.89 (0.69 – 1.15)	0.386	0.89 (0.58 – 1.37)	0.595
Tumour grade				
3 vs 1-2	1.64 (1.22 – 2.20)	0.001	1.69 (1.19 – 2.40)	0.004
Subtypes				
ER+PR+Her2-: Yes vs No ^a	0.63 (0.50 – 0.80)	<0.001		
ER+PR-Her2-: Yes vs No ^a	1.32 (0.99 – 1.75)	0.057		
ER+Her2+: Yes vs No ^{a,b}	0.63 (0.47 – 0.85)	0.002		
ER-Her2+: Yes vs No ^b	1.20 (0.95 – 1.53)	0.129		
Triple negative: Yes vs No	1.94 (1.46 – 2.32)	<0.001	1.16 (0.69 – 1.93)	0.577
Trastuzumab				
Yes vs No	0.81 (0.65 – 1.00)	0.052	0.76 (0.47 – 1.23)	0.263
Endocrine therapy				
Yes vs No	0.57 (0.47 – 0.69)	<0.001	0.55 (0.36 – 0.85)	0.007
Radiation therapy				
Yes vs No	1.09 (0.78 – 1.52)	0.626	0.74 (0.45 – 1.23)	0.251

^a Excluded from multivariable analysis due to collinearity with endocrine therapy. ^b Excluded from multivariable analysis due to collinearity with trastuzumab.

Abbreviations: HR Hazard ratio, ypNo axillary pathologic complete response, ypN1c/mi axillary residual isolated tumour cells or micrometastases, ypN1-3 axillary residual macrometastases, ypT-stage pathologic tumour stage after neo-adjuvant chemotherapy, ER estrogen, PR progesterone, Her2 human epidermal growth factor receptor 2.

Multivariable Cox regression analyses demonstrated no significant difference in OS between ypNo and ypN1c/mi (HR: 0.92 (0.27–3.09), $P=0.889$), but a significant difference in OS between ypNo and ypN1-3 (HR 1.70 (1.07–2.71), $P=0.026$) (Table 8.3).

Other independent predictors of decreased OS were higher ypT stage ((ypT 1-2: HR 2.40 (1.32–4.36), $P=0.004$) and (ypT 3-4: HR 4.38 (2.37–8.12), $P<0.001$)) and higher tumour grade (HR 1.72 (1.25–2.36), $P=0.001$). Furthermore, endocrine therapy (HR 0.49 (0.34–0.72), $P<0.001$) was identified as an independent predictor of increased OS.

Table 8.3 Uni- and multivariable analyses of predictors for overall survival.

	Univariable analysis		Multivariable analysis	
	HR (95%CI)	P-value	HR (95%CI)	P-value
ypNo	Reference		Reference	
ypN1tc/mi	1.07 (0.59 – 1.94)	0.816	0.92 (0.27 – 3.09)	0.889
ypN1-3	2.07 (1.62 – 2.67)	<0.001	1.70 (1.07 – 2.71)	0.026
Age (per year increment)	1.02 (1.02 – 1.03)	<0.001	1.01 (1.00 – 1.02)	0.082
ypT-stage				
To or Tis	Reference		Reference	
T1-2	1.60 (1.19 – 2.15)	0.002	2.40 (1.32 – 4.36)	0.004
T3-4	3.33 (2.41 – 4.59)	<0.001	4.38 (2.37 – 8.12)	<0.001
Tumour type				
Ductal	Reference		Reference	
Lobular	0.98 (0.72 – 1.33)	0.906	1.33 (0.78 – 2.27)	0.288
Other	0.98 (0.78 – 1.24)	0.882	1.03 (0.69 – 1.53)	0.887
Tumour grade				
3 vs 1-2	1.84 (1.41 – 2.40)	<0.001	1.72 (1.25 – 2.36)	0.001
Subtypes				
ER+PR+Her2-: Yes vs No ^a	0.62 (0.51 – 0.76)	<0.001		
ER+PR+Her2-: Yes vs No ^a	1.28 (1.00 – 1.65)	0.050		
ER+Her2+: Yes vs No ^{a,b}	0.58 (0.43 – 0.73)	<0.001		
ER+Her2+: Yes vs No ^b	1.15 (0.93 – 1.42)	0.201		
Triple negative: Yes vs No	2.10 (1.73 – 2.56)	<0.001	1.38 (0.90 – 2.13)	0.145
Trastuzumab				
Yes vs No	0.72 (0.59 – 0.88)	0.001	0.70 (0.46 – 1.07)	0.101
Endocrine therapy				
Yes vs No	0.56 (0.47 – 0.66)	<0.001	0.49 (0.34 – 0.72)	<0.001
Radiation therapy				
Yes vs No	1.31 (0.96 – 1.77)	0.086	1.06 (0.64 – 1.74)	0.829

^a Excluded from multivariable analysis due to collinearity with endocrine therapy. ^b Excluded from multivariable analysis due to collinearity with trastuzumab.

Abbreviations: HR Hazard Ratio, ypNo axillary pathologic complete response, ypN1tc/mi axillary residual isolated tumour cells or micrometastases, ypN1-3 axillary residual macrometastases, ypT-stage pathologic tumour stage after neo-adjuvant chemotherapy, ER estrogen, PR progesterone, Her2 human epidermal growth factor receptor 2.

Discussion

This is the first study comparing prognosis of ypNo with ypN1tc/mi and ypN1-3 in cN+ breast cancer patients treated with NAC. It is well known that axillary pCR is an important prognostic factor.⁶⁻⁸ Residual axillary disease after completion of NAC is associated with a less favourable prognosis. However, to our knowledge, this is the first study that compares the long-term effect of different degrees of residual disease on prognosis. Our study showed that ypNo and ypN1tc/mi carry similar prognosis and that ypN1-3 carries a significantly different and less favourable prognosis in terms of DFS and OS.

Current guidelines still recommend to perform ALND in cN+ patients following NAC irrespective of axillary response.^{10,11} However, cN+ patients converting to axillary pCR after completion of NAC remain a topic of debate since they are not expected to benefit from ALND. A non-invasive technique to accurately diagnose pCR is currently unavailable. Various minimally invasive procedures have been suggested for this purpose. The SLNB was studied extensively and its reliability seems questionable with a reported overall false negative rate (FNR) of 15.1% and negative predictive values (NPV) of 86% or lower.⁵ Other recently introduced minimally invasive techniques, the MARI procedure (Marking the Axillary lymph node with Radioactive Iodine seeds) and TAD (Targeted Axillary Dissection), are promising with FNRs of 7 and 2%, respectively. However, with only evidence available of single center studies comprising small cohorts that support these techniques it is not (yet) safe to implement them in clinical practice.^{12,13}

In our cohort, all patients underwent an ALND and thus our results do not directly support a change in surgical axillary treatment after the completion of NAC. Considering the comparable prognosis between ypNo and ypNite/mi, our results do question whether ypNite/mi may mimic ypNo more than residual axillary disease. Thus, when minimally invasive procedures prove to predict the status of the axilla accurately, the indications for omitting ALND may not just be limited to ypNo. Therefore, current research on reducing axillary management in cN+ patients should not focus only on ypNo patients, but also on patients with ypNite/mi. In future, ALND may be rendered as a procedure only to manage residual macrometastases.

In clinically node-negative patients in adjuvant setting, the SLNB with a relatively high FNR of about 8% is permitted since axillary recurrences are rare and previous studies have shown that not all axillary residual disease eventually converts to clinically overt disease.^{2,3,14} This is in part effectuated by adjuvant therapy (i.e., radiation and/or systemic therapy) and by biological subtypes influencing recurrence patterns. In cN+ patients, however, no studies have adequately evaluated prognostic impact of omitting ALND in case of residual axillary disease. Despite this, a trend toward replacing ALND by less invasive axillary staging procedures that are known to miss potentially therapy-resistant disease is already ongoing worldwide. Therefore, it is of utmost importance to prospectively collect data of these patients to detect potential influences on prognosis.

Since prognosis seems comparable between post-ALND ypNo and ypNite/mi in cN+ patients treated with NAC, imaging might play an important role in axillary staging after NAC in the future. Since ITCs and micrometastases are not detectable on high-resolution exams, such as MRI or 18F-FDG PET/CT, imaging techniques were considered inaccurate for nodal assessment after completion of

NAC. Yet, with our current observations in mind, dedicated axillary imaging is re-entering the arena as a modality to non-invasively identify residual macrometastases rather than ‘any’ extent of residual disease (including ITCs and micrometastases).

The strength of the current study is the large cohort of patients that all underwent ALND after NAC. But our study also has several limitations. Subgroups ypNo and ypNi-3 comprised 299 and 997 patients, respectively, where subgroup ypNItc/mi comprised only 51 patients. Our ypNItc/mi subcohort was too small to explore the influence of single versus multiple tumour-positive lymph nodes on prognosis, and further studies are needed to explore this concept. Yet, this subset of patients can be considered unique since ypNItc/mi in cN+ breast cancer is rare and a previously reported study included only a few ypNItc/mi patients.¹⁵

Furthermore, our cohort was treated up to a decade ago. In that time frame, different guidelines were effective, and therefore results should be interpreted carefully regarding current practice. For example, some Her2+ patients did not receive trastuzumab in our cohort (19.6%), since trastuzumab was just introduced by that time.

Finally, our results are based on a retrospective study design. Consequently, details on additional radiation therapy could not be taken into account since radiation therapy fields were not recorded for each patient. Therefore, its influence on prognosis could not be explored in more detail.

In conclusion, our study showed that prognosis of cN+ patients who receive NAC is affected by the degree of axillary residual disease as measured in ALND specimens. Prognosis of isolated tumour cells and micrometastases was comparable to prognosis of ypNo and more favorable than prognosis of macrometastases in terms of DFS and OS irrespective of tumour type. Ongoing and future studies should therefore consider ypNo and ypNItc/mi as one entity. Future research must explore which patients may safely receive a different, less invasive approach than the current standard of performing ALND after completion of NAC in all patients that were cN+ prior to NAC.

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Chapter 9

Does the TNM classification of solitary internal mammary lymph node metastases in breast cancer still apply?

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Abstract

Purpose

TNM classification of solitary internal mammary lymph node metastases (IMLNMs) in breast cancer varies depending on their method of detection: sentinel lymph node biopsy (pN1b) or clinical examination including radiological and/or physical examination (pN2b). This study aimed to evaluate whether there is a difference in prognosis between both groups.

Methods

Data of all patients diagnosed with primary invasive epithelial breast cancer between 2005 and 2008 were obtained from the Netherlands Cancer Registry. Patients with IMLNMs were divided in groups according to their pN1b and pN2b status. The main outcome measures disease-free survival (DFS) after 5 years and overall survival (OS) after 8 years were analyzed using Kaplan–Meier survival analysis. Cox regression analysis was used to determine independent predictors for DFS and OS.

Results

A total of 73 patients with pN1b status and 28 patients with pN2b status were included. DFS rate was 74.1% in the pN1b group compared to 85.0% in the pN2b group ($P=0.211$). Regarding OS, 20.5% (pN1b) and 25.0% (pN2b) of the patients deceased within 8 years of follow-up ($P=0.589$). In multivariable cox regression analysis, nodal status was not statistically significant for DFS (HR 0.29 (95% CI 0.04–2.33), $P=0.244$) or OS (HR 1.04 (95% CI 0.37–2.89), $P=0.947$).

Conclusions

Although the TNM classification considers pN1b and pN2b to be distinct prognostic entities, we did not observe any prognostic differences between these groups. Therefore, solitary IMLNMs may be regarded as a single category in the future and revision of TNM classification should be considered.

Introduction

In breast cancer staging, TNM classification is used to determine the anatomic extent of the disease and consequently identify specific subgroups with different prognoses.^{1,2} Pathologic nodal staging is an important element in this classification as the presence of regional nodal metastases is associated with impaired survival.³ These metastases can occur not only in axillary but also in extra-axillary lymph nodes, such as intramammary, periclavicular, interpectoral, and internal mammary lymph nodes.

Pathological nodal staging of internal mammary lymph node metastases (IMLNMs) has changed over time. In the fourth (1987) and fifth edition (1997) of TNM classification, all IMLNMs were classified as pN3, because by that time IMLNMs were considered of great importance in formulating the prognosis of patients.^{4,5} Since the introduction of the sixth edition in 2002, IMLNMs are divided into pN1b, pN1c, pN2b, or pN3b status depending on their method of detection and possible concurrent axillary lymph node metastases.^{6,7} IMLNMs may be detected by physical and/or radiological examination or by sentinel lymph node biopsy (SLNB).⁸ Nowadays, solitary IMLNMs, in the absence of axillary lymph node metastases, are considered pN1b when detected at SLNB and pN2b when detected at clinical examination (including physical and/or radiological examination).^{7,9,10}

Dividing solitary IMLNMs based on the method of detection, TNM implies a difference in prognosis between both groups. Therefore, the aim of this study was to evaluate whether a true difference in prognosis exists between pN1b and pN2b status.

Methods

Data collection

Data of all patients diagnosed between 2005 and 2008 with primary invasive epithelial breast cancer were obtained from the Netherlands Cancer Registry (NCR), which is managed by the Netherlands Comprehensive Cancer Organisation (IKNL). The NCR ensures a high-quality data collection using specially trained employees who extract patient, tumour, and treatment characteristics directly from the patient records. Groups were defined according to pN1b (IMLNMs detected at SLNB) and pN2b (IMLNMs detected at clinical examination) nodal status. Characteristics collected were age, tumour characteristics (size, location, stage, grade, subtype, and receptor status), and

treatment characteristics (adjuvant chemotherapy, targeted therapy, endocrine therapy, and radiation therapy).

Treatment

During the study period, the Dutch national guideline of 2005 was in use.¹¹ This guideline recommended regional treatment depending on nodal status: SLNB was indicated in clinically node-negative patients, based on physical examination, with axillary ultrasound being commonly used but not mandatory at that time. Clinically node-positive (N+) patients, patients with positive SLNB, or patients with a contraindication for SLNB underwent axillary lymph node dissection (ALND).

In all patients who underwent lumpectomy, whole-breast irradiation was indicated. After mastectomy, chest wall irradiation was indicated in the case of irradical resection, pT4 tumours, and involvement of the pectoral muscle or skin. For pT3 tumours, chest wall irradiation was considered individually. Irradiation of regional nodal fields was included in case of four or more axillary lymph node metastases or involvement of top axillary lymph nodes after ALND. The recommended dose was 45–50 Gy in 5 weeks, with a boost to 60–70 Gy when residual tumour was present.

Chemotherapy was recommended in all premenopausal N+ women and in postmenopausal N+ women aged 50–69 with estrogen receptor (ER)- and progesterone receptor (PR)-negative tumours. Furthermore, chemotherapy was considered in physically fit postmenopausal N+ women aged 50–59 with ER- and PR-positive tumours and in N+ women aged 60–69 if four or more regional lymph nodes were involved. Chemotherapy regimen consisted of five courses of 5-Fluorouracil, Epirubicin, Cyclophosphamide (FEC) or six courses of Taxotere, Adriamycin, and Cyclophosphamide (TAC). Targeted therapy (trastuzumab) was recommended in selected cases in addition to chemotherapy in case of human epidermal growth factor 2 receptor amplification (HER2+). Endocrine therapy was recommended for all ER- and/or PR-positive tumours.

Statistical analysis

All analyses were performed using IBM SPSS Statistics version 23.0 (IBM Corporation, Armonk, New York, USA) and *P* values <0.05 were considered statistically significant. Differences between pN1b and pN2b groups with regard to patient, tumour, and treatment characteristics were tested using the Fisher's Exact Test and Pearson Chi-square test for categorical variables and Mann-Whitney U test for continuous variables.

The main outcome measures were disease-free survival (DFS) after 5 years and overall survival (OS) after 8 years. DFS was defined as the absence of any first

local, regional, or contralateral recurrence, distant metastasis, or mortality within 5 years. DFS rate included all patients without any event, who visited the hospital in the fifth year after diagnosis for regular check-up. OS was defined as the time interval between date of diagnosis and date of death or date of emigration, as obtained from the Municipal Personal Records Database and completed until December 31, 2014. Patients were censored at the date of their first event, date of last follow-up, date of death, or date of emigration, whatever came first. Patients without follow-up data were excluded from DFS analysis. DFS and OS were analyzed using Kaplan–Meier survival analysis and compared with the log-rank test.

Univariable and multivariable Cox regression analyses were used to determine relevant predictors for DFS and OS. Outcome measure was hazard ratio (HR) with corresponding 95% confidence intervals (CI). Due to the limited number of events, multivariable cox regression could only be performed with a limited number of variables.¹² Nodal status together with the most significant variables in univariable cox regression was selected for multivariable cox regression.

Results

General characteristics

Between 2005 and 2008, a total of 51,239 patients were diagnosed with primary invasive epithelial breast cancer. After selection for pN1b ($n=73$, 72.3%) and pN2b ($n=28$, 27.7%) status, a total of 101 patients remained, comprising 0.2% of the total population (Figure 9.1).

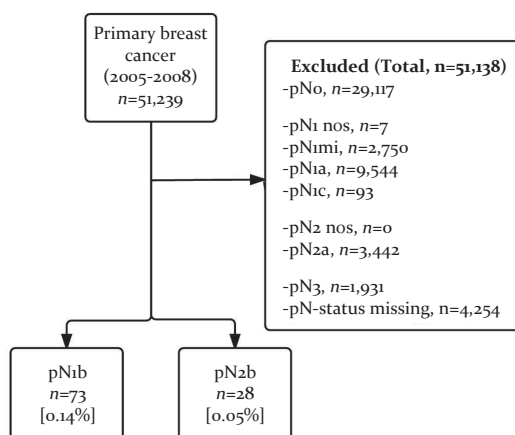


Figure 9.1 Flowchart of patient selection. Abbreviations: *nos* not otherwise specified, *mi* micrometastases, *pN3* includes pN3a, pN3b, and pN3c.

In comparison to pN1b status, pN2b was associated with lower rates of pT0-1 stage carcinoma (32 vs. 59%, $P=0.016$), lower rates of grade 1-2 carcinoma (32 vs. 63%, $P=0.005$), and larger mean tumour size (28 vs. 20 mm, $P=0.008$). A detailed overview of baseline patient, tumour, and treatment characteristics is shown in Table 9.1.

Table 9.1 Patient demographics and characteristics of tumour and treatment subdivided according to pN1b and pN2b status.

	pN1b (n=73)	pN2b (n=28)	<i>P-value</i>
Mean age, years (SD)	55 (14)	58 (17)	0.693
Mean tumour size, mm (SD)	20 (11)	28 (15)	0.008
pT-stage, n (%)			
To-1	43 (59)	9 (39)	0.016
T2-4	30 (41)	14 (61)	0.419
Unknown	-	5	-
Tumour type, n (%)			
Ductal	54 (74)	19 (68)	0.539
Lobular	7 (10)	4 (14)	0.492
Mixed ductal & lobular	4 (5)	3 (11)	0.393
Other	8 (11)	2 (7)	0.722
Grade, n (%)			
1-2	46 (67)	9 (38)	0.005
3	23 (33)	15 (62)	0.040
Unknown	4	4	-
Receptor Status, n (%)			
ER+, PR+, HER2-	39 (56)	16 (63)	0.737
ER+, PR-, HER2-	8 (11)	3 (11)	1.000
ER+, HER2+	9 (13)	3 (11)	1.000
ER-, HER2+	3 (4)	3 (11)	0.344
Triple negative	11 (16)	1 (4)	0.171
Unknown	3	2	-
Chemotherapy, n (%)	44 (60)	17 (61)	0.968
Radiation therapy, n (%)	55 (75)	19 (68)	0.447
Trastuzumab, n (%)	13 (18)	3 (11)	0.546
Endocrine therapy, n (%)	51 (70)	19 (68)	0.845

Abbreviations: SD Standard Deviation, *pT-stage* pathologic tumour stage, *ER* Estrogen Receptor, *PR* Progesterone Receptor, *HER2* Human epidermal growth factor receptor 2.

Disease-free survival (DFS)

Five-year follow-up data were complete for 54 patients (74.0%) in the pN1b group and 20 patients (71.4%) in the pN2b group. An event occurred in 13 patients (24.1%) in the pN1b group compared to two patients (10.0%) in the pN2b group ($P=0.211$) (Figure 9.2A). DFS rate was 74.1% in the pN1b group and 85.0% in the pN2b group. When taking the effect of endocrine therapy and triple-negative subtype into account in multivariable Cox regression analysis, pN2b status was

not significantly different compared to pN1b status (HR 0.29 [95% CI 0.04–2.33], $P=0.244$). Neither endocrine therapy nor triple-negative subtype was identified as an independent predictor for improved DFS (HR 0.46 [95% CI 0.12–1.86], $P=0.277$ and HR 1.56 [95% CI 0.35–7.06], $P=0.561$, respectively) (Table 9.2).

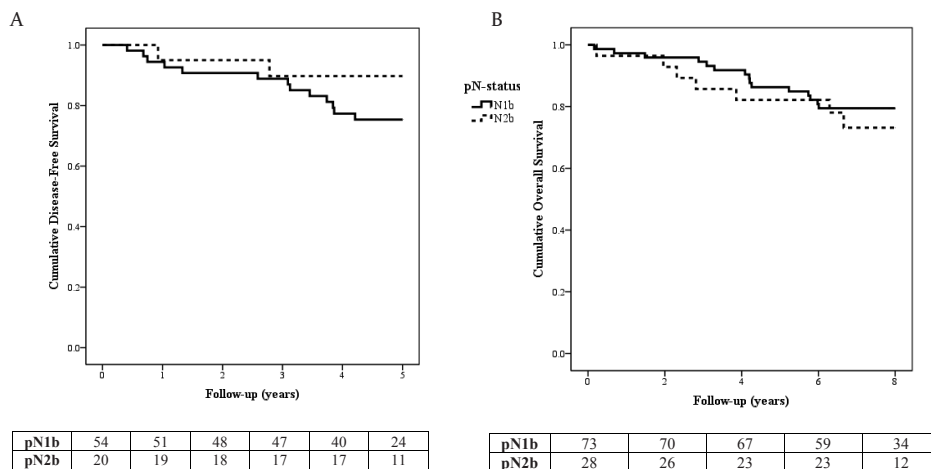


Figure 9.2 Kaplan–Meier survival curves of disease-free survival (A) and overall survival (B).

Table 9.2 Univariable and multivariable Cox Regression Analysis for Disease Free Survival.

	Univariable Cox Regression		Multivariable Cox Regression	
	HR [95% CI]	P-value	HR [95% CI]	P-value
pN1b	Reference		Reference	
pN2b	0.40 [0.09 – 1.77]	0.227	0.29 [0.04 – 2.33]	0.244
Tumour size (Per mm increment)	1.04 [1.00 – 1.07]	0.051		
pT-stage T2–4 vs T0–1	1.96 [0.71 – 5.42]	0.194		
Tumour grade 3 vs 1–2	1.07 [0.37 – 3.09]	0.897		
Triple negative subtype Yes vs No	3.58 [1.10 – 11.63]	0.034	1.56 [0.35 – 7.06]	0.561
Radiation therapy Yes vs No	1.13 [0.36 – 3.54]	0.838		
Chemotherapy Yes vs No	1.21 [0.44 – 3.35]	0.709		
Endocrine therapy Yes vs No	0.25 [0.09 – 0.70]	0.008	0.46 [0.12 – 1.86]	0.277
Trastuzumab Yes vs No	0.33 [0.04 – 2.47]	0.200		

Abbreviations: HR Hazard Ratio, CI Confidence Interval, pT-stage pathological tumour stage.

Overall survival (OS)

Median follow-up time of patients was 7.7 years (range 59 days–9.9 years). After 8 years of follow-up, 15 patients (20.5%) in the pN1b group and seven patients (25.0%) in the pN2b group were deceased ($P=0.589$) (Figure 9.2B). When taking the effect of tumour size (per mm increment), endocrine therapy, and trastuzumab into account in multivariable Cox regression analysis, pN2b status still was not significantly different compared to pN1b status (HR 1.04 [95% CI 0.37–2.89], $P=0.947$). Tumour size (HR 1.02 [95% CI 1.00–1.05], $P=0.117$), endocrine therapy (HR 0.40 [95% CI 0.15–1.04], $P=0.060$), and trastuzumab (HR 0.26 [95% CI 0.04–1.98], $P=0.192$) did not have a statistically significant influence on OS (Table 9.3).

Table 9.3 Univariable and multivariable Cox Regression Analysis for Overall Survival.

	Univariable Cox Regression		Multivariable Cox Regression	
	HR [95% CI]	<i>P</i> -value	HR [95% CI]	<i>P</i> -value
pN1b	Reference		Reference	
pN2b	1.28 [0.52 – 3.14]	0.590	1.04 [0.37 – 2.89]	0.947
Tumour size				
(Per mm increment)	1.04 [1.01 – 1.06]	0.003	1.02 [1.00 – 1.05]	0.117
pT-stage*				
T2-4 vs T0-1	2.19 [0.91 – 5.29]	0.082		
Tumour grade				
3 vs 1-2	1.73 [0.67 – 4.49]	0.259		
Triple negative subtype				
Yes vs No	2.22 [0.74 – 6.71]	0.156		
Radiation therapy				
Yes vs No	0.85 [0.31 – 2.30]	0.748		
Chemotherapy				
Yes vs No	1.06 [0.45 – 2.48]	0.897		
Endocrine therapy				
Yes vs No	0.30 [0.13 – 0.69]	0.005	0.40 [0.15 – 1.04]	0.060
Trastuzumab				
Yes vs No	0.22 [0.03 – 1.63]	0.138	0.26 [0.04 – 1.98]	0.192

Abbreviations: HR Hazard Ratio, CI Confidence Interval, pT-stage pathological tumour stage. * Excluded from multivariable analysis due to collinearity with tumour size.

Discussion

According to the current TNM classification, patients with solitary IMLNMs are considered pN1b when detected during SLNB and pN2b when observed during clinical examination (including radiologic and/or physical examination), suggesting a prognostic difference between these two groups.^{1,2,6} However, our study demonstrated that both DFS after 5 years ($P=0.211$) and OS after 8 years

($P=0.589$) were not significantly different between both groups. Consequently, it is questionable whether the current TNM classification of IMLNMs is still appropriate.

The comparable prognosis of the pN1b and pN2b group in our study can be explained by the great improvements in imaging modalities over the last decade. In the past, clinical detection of IMLNMs was mostly restricted to large internal mammary lymph nodes found during physical examination (and later additional ultrasound if indicated).

Consequently, IMLNMs detected during physical examination were much larger and thus associated with worse prognosis than IMLNMs detected during SLNB. In distant past, 10-year overall survival ranged from 0 to 61% in patients with IMLNMs compared to our cohort of patients with SLNB-detected IMLNMs, of which only 20.5% of the patients deceased after 8 years of follow-up.^{6,13,14} Possible explanations for improved overall survival can be the introduction of other systemic regimen, such as trastuzumab, or detecting smaller IMLNMs with SLNB. Nowadays, the size of internal mammary lymph nodes detected using state-of-the-art imaging techniques such as PET/CT and MRI approaches the size of internal mammary nodes visualized during SLNB.¹⁵⁻¹⁷ This suggests comparable prognosis of SLNB-detected IMLNMs and imaging-detected IMLNMs.

Routine evaluation of IMLNMs is controversial and is currently not recommended. The overriding arguments against routine evaluation of IMLNMs include their low incidence, their very limited impact on prognosis and treatment strategy, and the fact that tissue sampling is rather complex and associated with a risk of morbidity.^{13,18,19} However, detection of IMLNMs during radiological examinations and SLNB will continue to occur and possibly even increase with improving accuracy of these techniques. Their unambiguous and accurate classification will remain important as the detection of IMLNMs may alter nonsurgical treatment in patients.²⁰ Current guidelines advise internal mammary irradiation in all patients with histologically proven and/or PET-positive IMLNMs and in patients with N2 status additional radiotherapy of the periclavicular region and/or thoracic wall can be advised.^{9,10,21} A previous study by Heuts et al. demonstrated that adjuvant treatment plans were changed in only 3.4% (27/789) of the patients based on the presence of IMLNMs.²⁰ If TNM classification would be adapted by including all isolated IMLNMs in one group, then additional radiation therapy, besides internal mammary irradiation, could be omitted in these patients.

A major strength of this retrospective study is the use of a large population-based dataset from the Netherlands Cancer Registry providing patient, tumour, and treatment characteristics. However, as metastatic spread to the internal mammary lymph node chain is rare, a limited number of patients were only available per subgroup.²² Early surgical series showed internal mammary

involvement in 9.1% of patients undergoing extended radical mastectomy.²³ According to our study, solitary IMLNMs were reported in only 0.2% of the population suggesting that IMLNMs may currently be underdiagnosed. Firstly, routine evaluation of IMLNMs is not recommended. Secondly, according to literature, superficial tracer injection (intradermal or periareolar) often used during SLNB yields a lower visualization rate of internal mammary sentinel lymph nodes compared to intraparenchymal tracer injection (peritumoural, intratumoural, or subtumoural).²⁴ All in all, the results of this study should be interpreted in the context of this small study population.

Furthermore, the staging technique used to classify patients as pN2b in our cohort was unknown. As a consequence, there was no distinction in our cohort of pN2b patients detected by for instance physical examination, ultrasound, MRI, or PET-CT. Yet, a previous study of Jochelson et al. demonstrated a difference in the prevalence between several imaging techniques for detecting internal mammary adenopathy.¹⁷

Another study limitation may be the completeness of data. Nodal status was missing in over 4000 patients (10.3%) of the overall population of patients diagnosed with breast cancer in the Netherlands between 2005 and 2008. However, subclassification of pN1 (into pN1mi, pN1a, pN1b, and pN1c) and pN2 (into pN2a and pN2b) status seems to be accurately registered as in the pN1 group only seven patients were classified as pN1 not otherwise specified and none in the pN2 group (Figure 9.1). Therefore, registration of nodal status in our cohort was performed adequately.

In conclusion, our study did not observe any difference in prognosis between pN1b and pN2b in terms of DFS and OS. Since coincidental detection of IMLNMs during SLNB and radiological examinations will continue to occur and possibly even increase with improving accuracy of these techniques, their unambiguous and accurate classification will remain important. Therefore, more research on pN1b and pN2b is advised and revision of TNM classification is desirable as solitary IMLNMs may be regarded as a single category.

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Chapter 10

Discussion and future perspectives

Discussion

The aim of this thesis was to investigate the enigma of lymph node staging in breast cancer patients, by improving diagnostic accuracy of nodal staging in order to achieve a more patient-tailored treatment with minimal invasive therapy and reduced morbidity. The first part concerns invasive lymph node staging techniques after completion of neoadjuvant systemic therapy: sentinel lymph node biopsy (SLNB) and RISAS-procedure (Radioactive Iodine Seed localization in the Axilla in axillary node positive breast cancer combined with Sentinel node procedure following neoadjuvant chemotherapy). The second part describes non-invasive lymph node staging techniques (axillary ultrasound (US), standard T2-weighted MRI (T2W-MRI), gadofosveset-enhanced axillary MRI (GDF-MRI), PET/CT and dedicated axillary hybrid PET/MRI). In the final part results concerning classification of lymph node metastases (infraclavicular lymph node metastases, axillary lymph node metastases after neoadjuvant systemic therapy and solitary internal mammary lymph node metastases) are reported.

Part I – Invasive lymph node staging

In clinically node negative patients, SLNB is generally accepted as standard axillary diagnostic technique for lymph node staging, with previously reported false-negative rate (FNR) of 4% and negative predictive value (NPV) of 96%.¹ In clinically node positive patients treated with neoadjuvant systemic therapy, there is currently many ongoing research in order to explore a less invasive technique as opposed to axillary lymph node dissection (ALND) to accurately identify axillary pCR to prevent patients of ALND morbidity. According to chapter 2, SLNB in clinically node positive patients treated with neoadjuvant systemic therapy is inaccurate for assessment of axillary pathologic complete response (pCR), with a pooled FNR of 15.1% and a NPV of 86% or less.² In subgroup analyses, FNR significantly improved in case two or more sentinel nodes were removed compared to only one (FNR 10.4% versus 23.9%, $P=0.026$). This might suggest SLNB can be used as less invasive diagnostic technique in clinical practice, as opposed to axillary lymph node dissection (ALND). However, according to the SENTINA-trial only 69% (156/226) of patients with residual axillary nodal disease had 2 or more sentinel nodes.³ Consequently, SLNB can be considered an inaccurate technique in these patients, since over 30% of patients with residual axillary nodal disease would be incorrectly diagnosed and consequently treated then.

Another less invasive technique to determine axillary pCR in clinically node positive patients after neoadjuvant systemic therapy was first described by Donker et al: the MARI-procedure (Marking the Axillary lymph node with Radioactive Iodine seeds).⁴ The MARI-procedure reported a promising FNR of 7%. Axillary pCR in the MARI node was identified in 30 patients. Five out of these 30 patients had residual tumour burden in the ALND and therefore NPV remained 83%.⁵ In other words, 1 out of 6 patients with negative MARI nodes will still have residual lymph node metastases in other axillary lymph nodes. Therefore, MARI-procedure as a solitary post-neoadjuvant systemic therapy axillary staging procedure may not seem sufficiently accurate for the prediction of axillary pCR. Consequently, MARI-procedure followed by axillary radiotherapy was suggested, depending on its response on imaging.⁶ Yet, previous studies already showed significantly more comorbidity once any axillary surgery was followed by axillary radiotherapy, compared to axillary surgery alone.⁷ A minimal invasive technique which can accurately assess the pathological status of remaining lymph nodes following neoadjuvant systemic therapy, without requiring adjuvant axillary radiotherapy, is therefore preferred.

An alternative technique was introduced by Caudle et al.: Targeted Axillary lymph node Dissection (TAD).⁸ In contrast to the MARI-procedure, an iodine seed was placed in the histopathologically confirmed axillary lymph node metastasis after completion of neoadjuvant systemic therapy. Recently, first results of the TAD-procedure were described in a cohort of 208 patients, of whom 85 patients underwent TAD-procedure and completion ALND. They reported promising results of FNR 2% (1/50) and NPV 97% (35/36).⁹ Limitations are the sample size of patients with axillary pCR in this cohort (n=36) and absence multicenter design of the study.

In summary, there is currently no less invasive procedure in clinical practice to accurately identify axillary pCR. However, the combination of both SLNB and MARI-procedure has the potential to discover small (SLNB) as well as larger nodal metastases (MARI). It certainly has the potential to outperform both SLNB and MARI-procedure alone. In chapter 3, a trial report of a new Dutch clinical prospective multicenter trial is described in detail, in which both SLND and MARI will be combined as one procedure: RISAS-procedure.¹⁰ For the next two years, a total of 225 patients will be included in this Dutch multicenter trial, in order to investigate whether the RISAS-procedure can truly identify axillary pCR accurately as compared to ALND.

Part II – Non-invasive lymph node staging

Current European guidelines recommend axillary ultrasound (US) for lymph node staging in every patient.¹¹ However, the role of US has become questionable since the ACOSOG Z0011 trial demonstrated that a completion ALND can be safely omitted in clinically node negative patients based on physical examination.¹² Yet, US is relevant though in selecting clinically node positive patients, who have a worse prognosis when compared to (clinically node negative) SLNB-positive patients.¹³ Consequently, excluding advanced axillary nodal disease (pN2-3) rather than detecting any positive lymph node is becoming more important. Previous studies reported probabilities of pN2-3 given node negative axillary US findings ranging from 4–7%.^{14,15} However, in case of suspicious nodal axillary findings, it cannot differentiate between limited axillary nodal disease (pN1) and pN2-3.¹⁴

The role of standard T2-weighted (T2W) breast MRI for axillary lymph node staging was investigated in chapter 4. After node negative findings on T2W-MRI, with a complete field of view of the axillary region, there is no further need for axillary US to safely exclude pN2-3.¹⁶ In case of node positive findings on T2W-MRI, it outperformed axillary US regarding the probability of pN2-3 given one to three suspicious nodes (29% on US versus 12–15% on MRI). Hence, standard T2W-MRI can be used to more accurately define lymph node status in clinically node positive patients.

In order to further optimize MRI for differentiating between node negative and positive findings, lymph node specific contrast agent (Gadofosveset) was suggested. Gadofosveset was formerly used as blood pool agent in MR-angiography.¹⁷ It is characterized by its binding to serum albumin, which is also present in lymph nodes. Hypothetically, concentration of albumin decreases in case of cancer invasion into lymph nodes, which allows differentiation between benign (high uptake of contrast agent) and malignant (low uptake of contrast agent) lymph nodes.¹⁸ In rectal cancer, the use of gadofosveset-enhanced MRI (GDF-MRI) improved diagnostic performance for lymph node staging.^{19,20} In breast cancer nodes, a feasibility study demonstrated promising results with GDF-MRI.²¹ Of special interest is the high NPV which even outperforms the NPV of SLNB (NPV 96%) suggesting that axillary surgery could be safely omitted in clinically node negative patients.¹ In chapter 5, the diagnostic performance of GDF-MRI was investigated in a larger cohort of 97 patients. The diagnostic performance of GDF-MRI, on a node-by-node analysis, was: sensitivity 59%, specificity 87%, positive predictive value 65% and NPV 84%. However, a learning curve existed and NPV improved in the expert GDF-reader from 79% to 92%, after reading 176 lymph nodes (AUC 0.95). Therefore, GDF-MRI could potentially be used in clinical practice, but unfortunately the manufacturer recently halted

production of gadofosveset. Consequently, our validation study was closed early and future use of the contrast-agent gadofosveset is insecure.

Besides MRI, Koolen et al. demonstrated that PET/CT can be considered of added value for lymph node staging in breast cancer with a positive predictive value of 98% for axillary lymph node metastases.²² Unfortunately, PET/CT suffered from a relatively low NPV of 53% in case of non-FDG avid lymph nodes. This indicates that the overall use of PET/CT is limited in clinical practice since axillary surgery in patients with negative findings on PET/CT cannot be safely omitted.^{22,23} Furthermore, the amount of FDG-activity on PET/CT, which is indicative to differentiate between benign and malignant findings, varies per subtypes. It is most accurate in estrogen positive (ER+) and Her2- or triple negative subtypes, in contrast to MRI which is most accurate in ER+/Her2+ and ER-/Her2+ subtypes.^{24,25}

A combination of both promising imaging modalities, i.e. MRI and PET, would be a next step to further optimize non-invasive lymph node staging: the accuracy to identify patients with axillary lymph node metastases by PET (high PPV) and the accuracy to exclude patients without axillary lymph node metastases by MRI (high NPV). After having overcome the technical hurdles, the combination of both imaging modalities was successfully achieved in 2008 by introducing a hybrid PET/MR system.²⁶ A previous study of Melsaether et al. reported an improved identification for axillary lymph node metastases with the use of PET/MRI compared to PET/CT, with an increased sensitivity from 88% to 100%.²⁷ However, this study only differentiated between negative and positive axillary lymph nodes rather than reporting the number of suspicious nodes. In current clinical practice, the number of suspicious nodes is important regarding adjuvant axillary treatment (i.e. adjuvant radiation therapy of periclavicular region and thoracic wall). In chapter 6, a pilot study demonstrated improved differentiation between limited and advanced axillary nodal disease with the use of a dedicated axillary hybrid PET/MR protocol compared to current imaging modalities like ultrasound, MRI and PET/CT. According to dedicated axillary hybrid PET/MRI findings, clinical nodal status changed in up to 75% of patients. Therefore, it was concluded that dedicated axillary hybrid PET/MR could have potential value in clinically node positive patients to improve differentiation between limited and advanced axillary nodal disease.

Part III – Classification of lymph node staging

The TNM classification system not only aims to provide an indication of prognosis, but also assists in treatment planning and evaluation of treatment effects.²⁸ In breast cancer, correct classification of lymph nodes has major consequences since treatment strategies are based on TNM classification. According to this thesis, reclassification of both solitary internal mammary lymph node metastases (pN1b/pN2b) and infraclavicular lymph node metastases (pN3a) can be advised. Solitary internal mammary lymph node metastases should be considered as a single category in future.²⁹ In addition, infraclavicular lymph node metastases, with a maximum of nine lymph node metastases in total, should be considered as pN2a rather than pN3a³⁰.

Furthermore, the impact of remaining nodal disease after completion of neoadjuvant systemic therapy was investigated. Our research showed that prognosis of axillary pCR and isolated tumor cells and/or micrometastases was comparable in clinically node positive patients treated with neoadjuvant systemic therapy followed by ALND.³¹ Therefore, small metastases seem less relevant compared to macrometastases. This supports the role of imaging after completion of neoadjuvant systemic therapy, since small metastases (i.e. isolated tumour cells/micrometastases) can be missed on imaging modalities without any prognostic consequences for the patient.

Future perspectives

Future studies should further explore the diagnostic performance of dedicated axillary hybrid PET/MRI for lymph node staging in breast cancer. Hypothetically, dedicated axillary hybrid PET/MRI could be used prior to surgery in any patient to evaluate potential presence of axillary lymph node metastases. The impact could be practice changing with better stratification of patients for axillary surgery. In addition, cost-effectiveness studies should be performed to investigate whether dedicated axillary hybrid PET/MRI can potentially serve as non-invasive lymph node staging tool to replace axillary surgery in clinically node negative patients. In clinically node positive patients, nodal response after neoadjuvant systemic therapy can potentially be assessed with dedicated axillary hybrid PET/MRI too. This allows clinically node positive patients who converted to axillary pCR after administration of neoadjuvant systemic therapy to omit axillary surgery and minimizing the risk of morbidity caused by surgery.

Conclusion and recommendations

The ongoing RISAS trial will answer the question whether RISAS-procedure is an accurate method for less invasive axillary lymph node staging as opposed to ALND in clinically node positive patients treated with neoadjuvant chemotherapy with the aim to accurately identify axillary pCR. Eventually, RISAS-procedure could prevent morbidity, by omitting ALND in patients with negative RISAS nodes.

Meanwhile, non-invasive lymph node staging will be further optimized to improve evaluation of lymph nodes after neoadjuvant systemic therapy. Determination of these lymph nodes should focus on residual macrometastases rather than any extent of nodal disease.

In clinical practice, axillary US only is sufficient in case of node negative findings treated with SLNB. Yet, T2W-MRI covering the entire axillary region can replace axillary US if the MR shows node negativity.

In clinically node positive patients, T2W-MRI is more accurate compared to axillary US to define the number of suspicious axillary lymph nodes. Dedicated axillary hybrid PET/MRI has potential to improve accuracy of lymph node staging, especially regarding the number of suspicious lymph nodes. Solid evidence has yet to be built and together with its limited availability only in research institutes this makes that PET/MRI is not yet ready for clinical practice.

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Summary

Summary

The aim of this thesis was to investigate the enigma of lymph node staging in breast cancer patients, by improving the diagnostic accuracy of nodal staging in order to achieve a more patient-tailored treatment with minimal invasive therapy and reduced morbidity.

Part I – Invasive lymph node staging

Currently, there is ongoing research exploring less invasive techniques as opposed to axillary lymph node dissection (ALND) to accurately identify axillary pathologic complete response (pCR) in clinically node positive patients treated with neoadjuvant systemic therapy, in order to prevent patients of ALND related morbidity. *Chapter 2* described a systematic review and meta-analysis of a total of eight studies investigating sentinel lymph node biopsy (SLNB) as less invasive technique as opposed to ALND. According to our results, SLNB is not able to accurately identify axillary pCR, with a pooled identification rate of 92.3%, pooled false-negative rate of 15.1% and negative predictive values ranging from 61.5% - 86.3%.

Chapter 3 reported the rationale and design of a Dutch prospective multicenter validation study: RISAS (Radioactive Iodine Seed localization in the Axilla with the Sentinel node procedure). For the next two years, this study will investigate the diagnostic accuracy of the RISAS-procedure in 225 clinically node positive patients treated with neoadjuvant systemic therapy.

Part II – Non-invasive lymph node staging

In *chapter 4*, standard breast MRI for axillary lymph node staging was compared to dedicated axillary ultrasound. Two independent breast radiologists reassessed breast MRI exams of 377 patients. The probability of pN2-3 given cNo for breast MRI and axillary ultrasound was 0.7-0.9% versus 1.5%. In case of cN1, probability of pN2-3 on breast MRI was 11.6-15.4% versus 29.0% on axillary ultrasound. It was concluded that evaluation of axillary nodal status on standard breast MRI is comparable to dedicated axillary ultrasound.

A validation study on gadofosveset-enhanced axillary MRI for nodal (re)staging was described in *chapter 5*. Ninety patients underwent T2 weighted- and gadofosveset-enhanced axillary MRI. Two independent readers assessed these

axillary MRI exams. After reading 176 nodes, sensitivity, specificity, PPV, NPV and AUC for reader 1 improved from 38%, 89%, 56%, 79% and 0.71 to 60%, 93%, 64%, 92% and 0.95. AUC of reader 2 improved from 0.69 to 0.79. Based on these results, gadofosveset-enhanced axillary MRI was considered to be a potential non-invasive imaging tool for nodal (re)staging in breast cancer.

A feasibility study investigating dedicated axillary 18F-FDG hybrid PET/MRI was reported in *chapter 6*. Twelve clinically node positive patients underwent axillary ultrasound and dedicated axillary PET/MRI. Nine of these twelve patients also underwent whole-body PET/CT. Regarding the number of suspicious axillary lymph nodes, dedicated axillary hybrid PET/MRI changed nodal status when compared to standard imaging modalities: 40% compared to axillary ultrasound, 75% compared to T2 weighted MRI, 40% compared to contrast-enhanced MRI and 22% compared to PET/CT. Therefore, dedicated axillary 18F-FDG hybrid PET/MRI was considered feasible and might improve diagnostic performance of axillary nodal staging in clinically node positive patients.

Part III – Classification of lymph node staging

In *chapter 7*, patients with a pN3a nodal status (i.e. at least one infraclavicular lymph node metastasis or \geq ten axillary lymph node metastases) were investigated, using data from the Netherlands Cancer Registry. According to the results from a cohort of 1,788 patients with pN3a, patients with \geq ten axillary lymph node metastases had a significantly worse prognosis compared to patients with at least one infraclavicular lymph node metastasis and nine or less lymph node metastases in total (disease-free survival (DFS): 48.8% versus 63.8%, $P=0.036$; overall survival (OS): 46.6% versus 63.9%, $P=0.042$). In addition, prognosis of patients with pN3a based on at least one infraclavicular lymph node metastasis was comparable to patients with pN2a (four to nine axillary lymph node metastases) (DFS: 63.8% versus 67.3%, $P=0.491$; OS: 63.9% versus 65.5%, $P=0.233$). Therefore, reclassification of infraclavicular lymph node metastases in the next TNM classification was suggested.

Chapter 8 compared prognosis of clinically node positive patients treated with neoadjuvant systemic therapy, subdivided by ypN status: axillary pCR (ypNo), residual axillary isolated tumour cells or micrometastases (ypN1c/mi) and residual axillary macrometastases (ypN1-3). In a cohort of 1,347 patients, obtained from data of the Netherlands Cancer Registry, similar prognosis was demonstrated between ypNo and ypN1c/mi (DFS: Hazard Ratio (HR) 1.38 (0.40-4.79), $P=0.613$; OS: HR 0.92 (0.27-3.09), $P=0.889$). Prognosis between ypNo and

ypN1-3 was statistically significant different (DFS: HR 1.78 (1.06-3.00), $P=0.031$; OS: HR 1.70 (1.07-2.71), $P=0.026$). In conclusion, future studies should consider ypNo and ypN1c/mi as one entity.

Besides axillary lymph node metastases, internal mammary lymph node metastases can reduce prognosis as well. Solitary internal mammary lymph node metastases, in the absence of axillary lymph node metastases, are divided in the current TNM classification according to method of detection: at SLNB (pN1b) or at clinical examination (pN2b). However, in *chapter 9* no prognostic difference was observed between both subgroups in a cohort consisting of 101 patients with solitary internal mammary lymph node metastases, obtained from data of the Netherlands Cancer Registry, after multivariable cox regression analyses (DFS: HR 0.29 (0.04-2.33), $P=0.244$; OS: HR 1.04 (0.37-2.89), $P=0.947$). Therefore, both subgroups may be regarded as a single category.

Samenvatting

Samenvatting

Het doel van dit proefschrift was het onderzoeken van lymfeklierstadiëring bij patiënten met borstkanker, door het verbeteren van diagnostische accuratesse op het gebied van lymfeklierstadiëring. Zodoende kan een meer patiëntgerichte behandeling uitgevoerd worden met minimaal invasieve therapie en verminderde kans op morbiditeit.

Deel I – Invasieve lymfeklierstadiëring

Momenteel zijn er verschillende studies die minder invasieve technieken in vergelijking tot een okselklierdissectie onderzoeken in klinisch klier-positieve patiënten behandeld met neoadjuvante systemische therapie. Deze techniek dient axillaire pathologisch complete respons (pCR) accuraat te kunnen identificeren. Indien een accurate techniek is vastgesteld, kan morbiditeit van een okselklierdissectie bij patiënten met axillaire pCR worden vermeden. *Hoofdstuk 2* onderzocht de schildwachtklierprocedure als minder invasieve techniek in vergelijking tot een okselklierdissectie bij klinisch klier-positieve patiënten behandeld met neoadjuvante systemische therapie, door middel van een systematisch review en meta-analyse van in totaal acht studies. Op basis van deze resultaten werd geconcludeerd dat de schildwachtklierprocedure geen accurate techniek is voor het identificeren van axillaire pCR, met een gepoolde identificatiewaarde van 92.3%, gepoolde fout-negatieve ratio van 15.1% en een negatief voorspellende waarde van 61.5% - 86.3%.

In *hoofdstuk 3* werd de achtergrond en methode van een Nederlandse prospectieve multicenter validatiestudie toegelicht: RISAS (lokalisatie van een axillair geplaatst radioactief jodiumzaadje in combinatie met de schildwachtklierprocedure). De komende twee jaar onderzoekt deze studie de diagnostische accuratesse van de RISAS-procedure in 225 klinisch klier-positieve patiënten behandeld met neoadjuvante systemische therapie.

Deel II – Non-invasieve lymfeklierstadiëring

Hoofdstuk 4 onderzocht de waarde van standaard MRI van de mammae voor axillaire lymfeklierstadiëring in vergelijking tot axillaire echografie. Twee onafhankelijke mamma-radiologen herbeoordeelden elk afzonderlijk 377 MRI-scans. De kans op pN2-3 in geval van cNo volgens MRI mammae en axillaire echografie was 0.7-0.9% versus 1.5%. In geval van cN1, was de kans op pN2-3

volgens MRI mammae was 11.6-15.4% versus 29.0% volgens axillaire echografie. Op basis van deze resultaten werd geconcludeerd dat evaluatie van axillaire lymfeklieren op MRI mammae vergelijkbaar is met echografie.

In *hoofdstuk 5* werd de waarde van gadofosveset-versterkte axillaire MRI voor (her)stadiëring van lymfeklieren onderzocht. Negentig patiënten ondergingen T2 gewogen en gadofosveset-versterkte axillaire MRI-scans. Twee onafhankelijke radiologen beoordeelden al deze MRI-scans. Na beoordeling van 176 lymfeklieren door de eerste beoordelaar, verbeterden sensitiviteit, specificiteit, positief voorspellende waarde, negatief voorspellende waarde en AUC van 38%, 89%, 56%, 79% en 0.71 naar 60%, 93%, 64%, 92% en 0.95. De AUC van de tweede beoordelaar verbeterde van 0.69 naar 0.79. Derhalve werd gadofosveset-versterkte axillaire MRI beschouwd als veelbelovend non-invasieve methode voor (her)stadiëring van lymfeklieren.

Een feasibility studie met ¹⁸F-FDG hybride PET/MRI specifiek gericht op de axilla werd beschreven in *hoofdstuk 6*. Twaalf klinisch klier positieve patiënten ondergingen axillaire echografie en axillaire hybride PET/MRI. Negen van de twaalf patiënten ondergingen tevens een PET/CT van het gehele lichaam. Axillaire hybride PET/MRI veranderde de klierstatus in vergelijking tot standaard beeldvormende technieken: 40% ten opzichte van axillaire echografie, 75% ten opzichte van T2 gewogen MRI, 40% ten opzichte van contrast-versterkte MRI en 22% ten opzichte van PET/CT. Concluderend, hybride PET/MRI specifiek gericht op de axilla is goed uitvoerbaar en zou de diagnostische performance van axillaire lymfeklierstadiëring kunnen verbeteren in klinisch klier-positieve patiënten.

Deel III – Classificatie van lymfeklierstadiëring

Hoofdstuk 7 onderzocht patiënten met een pN3a klierstatus (ten minste één infraclaviculaire lymfekliermetastase of \geq tien axillaire lymfekliermetastasen). Er werd gebruik gemaakt van data van het Integraal Kankercentrum Nederland (IKNL). In een onderzoekspopulatie van 1788 patiënten met pN3a werd aangetoond dat de prognose van patiënten met \geq tien axillaire lymfekliermetastasen significant slechter was in vergelijking tot patiënten met ten minste één infraclaviculaire lymfekliermetastase met in totaal \leq negen lymfekliermetastasen (ziektevrije overleving: 48.8% versus 63.8%, $P=0.036$; totale overleving: 46.6% versus 63.9%, $P=0.042$). Bovendien werd aangetoond dat de prognose van patiënten met ten minste één infraclaviculaire lymfekliermetastase vergelijkbaar is met patiënten met een pN2a klierstatus (vier tot negen axillaire lymfekliermetastasen) (ziektevrije overleving: 63.8% versus 67.3%, $P=0.491$; 63.9%

versus 65.5%, $P=0.233$). Revisie van de huidige classificatie van infraclaviculaire lymfkliermetastasen werd derhalve aanbevolen.

In *hoofdstuk 8* werd de prognose van klinisch klier-positieve patiënten behandeld met neoadjuvante systemische therapie onderzocht, waarbij er een onderverdeling werd gemaakt op basis van ypN status: axillaire pCR (ypNo), residuale axillaire geïsoleerde tumorcellen of micrometastasen (ypN1c/mi) en residuale macrometastasen (ypN1-3). In een cohort van 1347 patiënten, verkregen via het IKNL, werd een vergelijkbare prognose aangetoond tussen ypNo en ypN1c/mi (ziektevrije overleving: Hazard Ratio (HR) 1.38 (0.40-4.79), $P=0.613$; totale overleving: HR 0.92 (0.27-3.09), $P=0.889$). Prognose tussen ypNo en ypN1-3 was statisch significant verschillend (ziektevrije overleving: HR 1.78 (1.06-3.00), $P=0.031$; totale overleving: HR 1.70 (1.07-2.71), $P=0.026$). In conclusie, ypNo en ypN1c/mi zouden in toekomstige studies als één entiteit kunnen worden beschouwd.

Naast axillaire lymfekliermetastasen zijn parasternale lymfekliermetastasen prognostisch ongunstig. Geïsoleerde parasternale lymfekliermetastasen, dus in de afwezigheid van axillaire lymfekliermetastasen, zijn volgende de huidige TNM classificatie onderverdeeld op basis van methode van detectie: tijdens de schildwachtprocedure (pN1b) of tijdens klinisch onderzoek (pN2b). In *hoofdstuk 9* wordt echter geen prognostisch verschil gevonden tussen beide subgroepen, waarbij de onderzoekspopulatie bestaat uit 101 patiënten met geïsoleerde parasternale lymfekliermetastasen, verkregen via het IKNL. Na multivariabele cox regressieanalyses betreft ziektevrije overleving: HR 0.29 (0.04-2.33), $P=0.244$ en totale overleving: HR 1.04 (0.37-2.89), $P=0.947$. Derhalve zouden beide subgroepen wellicht als één categorie kunnen worden beschouwd.

Valorisation

Valorisation

Breast cancer is the most common type of invasive cancer among women worldwide, with an estimated 1.7 million cases and 521,900 deaths in 2012. In the Netherlands, the annual incidence is more than 14,000 patients. Nowadays, standard treatment consists of surgery of the breast and axilla, complemented with radiation and/or systemic therapy. This extensive treatment has led to a mean five year overall survival of 90%. Regarding the health care costs, breast cancer treatment represents approximately 15% of all health care costs in the Netherlands.

The excellent survival caused an attention shift from survival to the presence of morbidity and quality of life after treatment. Better patient selection for specific therapies and use of less invasive techniques, in order to avoid overtreatment on an individual level, aim to further minimize morbidity and complications of therapy and consequently reduce nationwide health care costs.

The aim of this thesis was to investigate the enigma of lymph node staging in breast cancer patients, by improving diagnostic accuracy of nodal staging in order to achieve a more patient-tailored treatment with minimal invasive therapy and reduced morbidity.

Relevance of scientific results in this thesis

Currently, axillary surgery is performed in every patient to investigate the axillary lymph node status. In clinically node negative patients sentinel lymph node biopsy (SLNB) is performed, whereas axillary lymph node dissection (ALND) is performed in clinically node positive patients. These procedures can cause complications, such as lymphedema, numbness and pain. In addition, patients without axillary lymph node metastases do not benefit from the axillary surgery, since they do not have any prognostic benefit from the procedure. Approximately 80% of clinically node negative patients and 40% of clinically node positive patients treated with neoadjuvant systemic therapy only have benign axillary lymph nodes. Yet, these patients are still exposed to axillary surgery with its concomitant complications.

This thesis explored further techniques to improve lymph node staging in breast cancer. We showed that patients with negative axillary findings on T2 weighted breast MRI, with a complete field of view of the axillary region, do not require any further diagnostic imaging. When compared to axillary ultrasound, T2

weighted breast MRI even improved differentiation between limited and advanced axillary nodal disease.

In clinically node positive patients treated with neoadjuvant systemic therapy, RISAS-procedure might become the new standard procedure to accurately identify axillary pCR. Previous studies already demonstrated less complications after SLNB as opposed to ALND. Consequently, ALND can be safely omitted in patients with axillary pCR according to RISAS, indicating these patients will experience less often any of the aforementioned surgical complications. Yet, final results of this Dutch prospective multicenter trials must confirm the validity of this technique first.

Finally, this thesis demonstrated that dedicated axillary ^{18}F -FDG hybrid PET/MRI is feasible in clinically node positive breast cancer patients to differentiate between limited and advanced axillary nodal disease. However, results of this technique should be further explored in research setting, before implementation into daily clinical practice is justified.

Target population

This thesis contributes to newly diagnosed breast cancer patients, by improving the diagnostic accuracy of axillary lymph node staging. In addition, results of this thesis are relevant for all physicians involved in treatment trajectories of breast cancer patients. Finally, dedicated axillary hybrid PET/MRI might encourage other researchers in the field of oncologic imaging in which lymph node staging is an important prognostic factor, such as melanoma, esophageal, ovarian, prostate or rectal cancer.

Innovation and future

Future studies should explore whether increasingly advanced imaging tools, like dedicated axillary hybrid PET/MRI, can accurately exclude lymph node metastases in clinically node negative patients, making SLND a redundant operation in node negative patients. Consequently, node negative patients would no longer harm from axillary surgery. In addition, cost-effectiveness of implementation of dedicated axillary hybrid PET/MRI should also be investigated.

Since the introduction a few years ago, hybrid PET/MRI has been incorporated in daily clinical practice for several (neurologic) indications. So far, no research has been performed investigating the feasibility of dedicated axillary hybrid PET/MRI to improve lymph node staging in breast cancer. This thesis demonstrated that dedicated axillary hybrid PET/MRI for lymph node staging is

feasible and it changed nodal status when compared to current imaging modalities.

Besides, diagnostic performance of dedicated axillary hybrid PET/MRI after neoadjuvant systemic therapy in clinically node positive patients should be investigated to determine whether this technique can accurately identify axillary pCR. This would allow patients who converted to axillary pCR to omit any further axillary surgery, which would be even more beneficial compared to RISAS-procedure.

